The Journal of Parasitology

Volume 24 AUGUST, 1938 Number 4

MAURICE CROWTHER HALL AS A PARASITOLOGIST

In a paper published in Science (1938, 87: 451–453), one of us (Schwartz) has given the essential facts regarding Dr. Hall's education, positions held by him, degrees and honors conferred upon him, and a general estimate of his personal traits and of his main work in parasitology. The facts already presented will not be repeated in this article which is an attempt to review briefly Dr. Hall's principal contributions to parasitology.

When Hall came to Washington in 1907 to join the staff of the Zoological Division of the U. S. Bureau of Animal Industry, the number of zoologists in this country who were primarily parasitologists could be counted on one's fingers. There was still much pioneer work to be done in the field of veterinary parasitology and Hall was eager and competent to do the pioneering. Although he had only one year's graduate training to his credit and was, therefore, not so well trained in parasitology as today's recruits, Hall was fortunate in having the unusual advantage of associating in Washington with such outstanding helminthologists as Charles Wardell Stiles, Brayton Howard Ransom and Nathan Augustus Cobb. Eager to make his mark in veterinary parasitology and endowed with a remarkable capacity for hard work, Hall became deeply engrossed in his investigations. In the course of his life he carried out a series of researches which were interrupted at first only by occasional and later by periodic illness to which he succumbed on May 1, 1938. In the review which follows, Dr. Hall's contributions to parasitology will be covered under the following headings: cestodes; nematodes; anthelmintics; and miscellaneous contributions.

CESTODES

Although as a graduate student in the University of Nebraska, Hall was interested in gregarines and published a paper on the genus *Hermocystis,* he apparently lost his interest in protozoology after taking up his work in Washington and reverted to this phase of parasitology only on rare occasions. His first problem in Washington involved investigations on the gid tapeworm, and he published a number of papers on this and related cestodes of carnivores. His most important work on the gid tapeworm from a zoological standpoint was a comprehensive historical

review of the literature pertaining to this and related species which he placed in the genus *Multiceps*. In 1910 he published a paper in which he made important suggestions for the control of the gid tapeworm which was then prevalent in Montana. This publication was designed to supply information to stockmen regarding the need of eradicating the gid tapeworm from sheep by prophylactic measures, such as burning the carcasses of dead sheep, and especially sheep heads, controlling the wanderings of dogs, eliminating vagrant dogs, and other control measures. As the result of his early work on the gid tapeworm, Hall developed a keen interest in carnivore tapeworms generally and this led to his publication of descriptions of new species of and of a synoptical key to the adult taeniaoid cestodes of dogs, cats, and other carnivores, and later to a comprehensive paper on the same subject published in 1919. The latter publication contains descriptions and illustrations of tapeworms occurring in carnivores and is still a standard reference to this subject.

NEMATODES

Dr. Hall's interest in nematodes was inevitable, considering the economic importance of these helminths and his own interest in problems which concerned the livestock industry of the United States. That he chose for his own investigations the nematodes of rodents rather than species of economically important host animals was purely accidental and due largely to the availability of a sizeable collection of nematodes of the orders RODENTIA, LAGOMORPHA, and HYRACOIDEA. This work led to the publication of an important monograph containing descriptions, illustrations, and keys to genera and species of nematodes from hosts of the orders mentioned; in addition, he did pioneer work in attempting to outline a general classification of nematodes and to define the superfamilies and families of this group. For a number of years following the publication of this monograph in 1916, Hall's paper was one of the standard treatises on nematodes and was used widely and advantageously by students and parasitologists.

ANTHELMINTICS

Dr. Hall's chief contribution to parasitology lies in the field of therapeutics. His first paper on the subject published in 1909 disproved De Renzi's claim that somatic taeniasis could be successfully treated with male fern. After the completion of his work on nematodes of rodents in 1915, the larger part of Hall's research work was carried out with the aim of improving anthelmintic medication and of discovering new and better anthelmintics and insecticides. During a period which extended from 1915 until his transfer from the Bureau of Animal Industry to the National Institute of Health in 1936, his publications on helminthology were incidental to his major researches on anthelmintics.

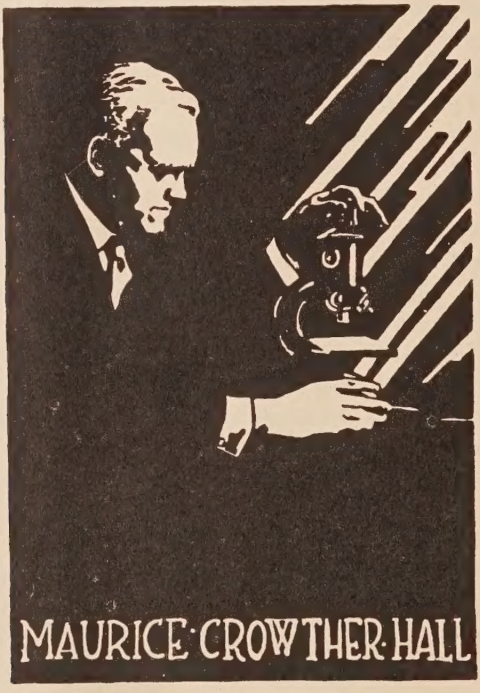
A paper by Hall and Foster in 1917 and several papers by Hall and his co-workers at Parke, Davis and Company which followed in the next year or two are, in a large measure, attempts to evaluate critically the many substances which were widely used at that time as anthelmintics. During the period that these investigations were in progress, the efficacy of anthelmintics was based almost solely on clinical experience with drugs, coupled with occasional observations that worms were passed by treated animals. Information based on the number of parasites *not* removed by treatment was generally not available. Hall insisted that such information was necessary to evaluate properly the efficacy of drugs used for removing worms.

Hall and his colleagues tested critically such diverse substances as the commonly used purges, including calomel, castor oil and magnesium sulphate; anesthetics, namely, chloroform and ether; antiseptics, namely iodoform and phenol; and miscellaneous substances which had been recommended as anthelmintics, including gasoline, petroleum, benzene, male fern, pelletierine tannate, areca nut, santonin, fig sap, spigelia, oil of chenopodium, kamala, and many others. Most of these substances proved of little value. Hall was able to condemn "stock tonics" and fluid extracts as useless and, at times, even as dangerous. His insistence on the importance of evaluating anthelmintics critically is one of Dr. Hall's most important contributions to the subject of therapeutics of parasitic infections. He developed the well-known critical test to supply the necessary information regarding the efficacy of anthelmintics, and in his papers he pointed out the limits of usefulness of this test. Without this critical test, Hall's demonstration of the anthelmintic efficacy of carbon tetrachloride could not have been established.

Hall and his co-workers determined that carbon disulphide was effective for the removal of bots from horses. Previous publications on the use of this compound in horses were based on clinical findings which, as Hall frequently reiterated, gave no information on the number of parasites not removed by the treatment employed and, therefore, no information regarding the injury from parasites still being sustained by the treated animal. At the time that Hall experimented with carbon disulphide it was generally believed that this compound was effective for the removal of "worms" from horses, the term "worms" being used in a nonspecific sense to include all verminous parasites of equines. Hall demonstrated that carbon disulphide was effective for the removal of ascarids from horses but that it was of little or no value for the removal of strongyles and other nematodes which commonly infect the cecum and colon of equines.

When Hall first made anthelmintic investigation his major interest, thymol and santonin had become almost unavailable because the supply

of these substances was cut off by the World War. The medical and veterinary professions of this country having been deprived of the anthelmintics which were commonly used at that time were turning enthusiastically to oil of chenopodium. Hall's search for effective anthelmintics naturally included tests with this drug. He was able to demonstrate that oil of chenopodium was superior to santonin for the removal of ascarids from swine, and at the present time there is no remedy superior to oil of chenopodium for the purpose indicated. It was generally believed at that



time that the palisade worms of horses were extremely difficult to remove. Only large and dangerous doses of anthelmintics then in use were thought to be effective for the removal of these parasites. Hall, Wilson and Wigdor demonstrated that suitable doses of oil of chenopodium were effective for the removal of strongyles from horses. Only in recent times have superior drugs for this purpose been discovered. The newer drugs used at present are halogenated hydrocarbons, the use of which may be traced directly to Hall's own work with substances belonging to this group of chemicals.

Hall tested oil of chenopodium critically in dogs (1918–1919) and

The book plate above, reproduced through the kindness of Mrs. Hall, was made by their daughter Marion in 1926.

found it to be the most effective drug for the removal of ascarids from these host animals. He demonstrated that chloroform was more effective for the removal of hookworms from dogs than oil of chenopodium and that these two drugs could be combined advantageously for the removal of ascarids and hookworms from these carnivore hosts.

In 1923 Hall and Shillinger tested arecoline hydrobromide critically as an anthelmintic for the removal of tapeworms from dogs. This drug had already been investigated by other workers and its efficacy was judged on the basis of clinical results. Hall and Shillinger demonstrated by critical tests that arecoline hydrobromide was generally effective for the removal of tapeworms from dogs, but that even in large doses it sometimes failed for unknown reasons.

The use of drugs as anthelmintics was only one of the angles of anthelmintic medication investigated by Hall. The methods of administration of anthelmintics were also points of intense interest to him and were subjected by him to critical investigations. Hall and his associates injected oil of chenopodium intravenously and intramuscularly in an attempt to destroy parasites present in locations other than the alimentary canal; this treatment was unsuccessful. Hall investigated the relative merits of such fine differences in method of administration as the use of hard gelatin capsules versus soft gelatin capsules. No point was too minute to attract his attention and no method of administration of anthelmintics was dismissed by him as worthless until it had been subjected to critical tests. His investigation of various methods of administration of anthelmintics bore positive results in at least one conspicuous case. In 1923 Hall and Shillinger demonstrated that the administration *per rectum* of oil of chenopodium and olive oil to chickens was an effective method of removing *Heterakis*. No other treatment which approaches this one in degree of efficacy has been discovered since that report was published.

Hall's greatest contribution to the therapeutics of verminous diseases was the discovery of the anthelmintic efficacy of carbon tetrachloride and his subsequent study of related compounds, some of which were found by him and others to possess anthelmintic efficacy. Because of the proven efficacy of chloroform, a compound closely related to carbon tetrachloride, Hall decided that the latter substance might be of value as an anthelmintic. He tested carbon tetrachloride in dogs and discovered in 1921 that this compound was very effective for the removal of hookworms from these host animals. He also suggested that carbon tetrachloride might be valuable for the removal of hookworms from man, and this drug was rapidly taken up by the medical and veterinary professions as a treatment for the removal of hookworms and found to be highly effective and much safer than any drug previously used for this purpose.

In 1921 Hall pointed out the probability that carbon tetrachloride would prove to be injurious to the liver, although he had no definite information on this point. Subsequent investigations by several workers have demonstrated that carbon tetrachloride produces serious damage to the liver. In fact, injury to the liver seems to be the first step in a series of reactions which may lead to serious and even fatal consequences when carbon tetrachloride is administered to human beings. Cases of carbon tetrachloride intoxication in man are apparently rare, however, since this chemical remained the drug of choice for the removal of hookworms from man for a number of years following its introduction, and was displaced only by tetrachlorethylene, a drug also introduced into human and veterinary medicine by Hall.

In his studies on carbon tetrachloride as an anthelmintic Hall pointed out that the superiority of carbon tetrachloride over chloroform might be due to the fact that the former contained an extra chlorine atom in each molecule; Caius and Mhaskar independently came to the same conclusion. The latter workers, however, did not submit their theory to a critical test. To Hall's mind a theory was of value only if it led to new discoveries. Accordingly, he tested the idea of the relation between chemical composition and anthelmintic efficacy by critical experiments. Hall and Cram tested carbon trichloride (hexachlorethane) for the removal of hookworms and ascarids from dogs with negative results. Hall and Shillinger tested ethylene dichloride as an anthelmintic with negative results; they reported, however, that tetrachlorethylene was even more effective against hookworms in dogs than carbon tetrachloride. The results obtained in this series of tests indicated that there was no relation between anthelmintic efficacy and the number of chlorine atoms in the molecule.

In discovering the anthelmintic value of tetrachlorethylene, Hall obtained an effective hookworm remedy which lacked the toxicity that carbon tetrachloride possesses, but was equally or even more effective as an anthelmintic. In tetrachlorethylene Hall found a safe and effective remedy for the removal of hookworms from man and animals. On the basis of the new evidence obtained, Hall hypothesized that the water solubility of an anthelmintic was correlated with its effectiveness. The extensive paper by Wright and Schaffer in 1932 demonstrated that the theory of the relationship between water solubility and anthelmintic efficacy was valid. The theory of the importance of water solubility in anthelmintic efficacy was corroborated by Lamson and his coworkers, who used another series of compounds, namely, the alkyl-phenols. Other workers have apparently utilized the theory of water solubility in developing treatments for the removal of helminths.

It is inevitable that even the most careful investigator will occasionally

find himself in error. It is surprising that among Hall's numerous investigations on anthelmintics one can find only one serious error. In 1926 Hall and Shillinger reported that kamala was a satisfactory anthelmintic for the removal of tapeworms from poultry. As poultry in the United States are commonly infected with tapeworms, kamala was widely exploited and extensively used. Rebrassier was unable to confirm Hall's results and he suggested a source of error in the critical tests used by Hall and Shillinger. When Rebrassier's work was confirmed in the Zoological Division, Hall reversed his former stand and testified in a court case on behalf of the Government that kamala was not effective for the removal of tapeworms from chickens, and he did not hesitate to admit his own error.

MISCELLANEOUS INVESTIGATIONS

During his fruitful life Hall explored many aspects of parasitology not as yet mentioned in this review. To enumerate all of his miscellaneous publications would involve the compilation of a sizeable article. Consequently, only a few of his more important papers in this field will be mentioned. Hall was a pioneer in the study of methods of examining feces for evidence of parasitic infection. In 1911 he published an extensive paper on this subject in which he reviewed all of the methods known at that time and proposed a new method which he had developed. Although the method proposed by Hall is no longer used because of improved technique developed in recent years, his paper on the subject is still of interest to parasitologists because of its historical value.

In the field of veterinary parasitology a knowledge of the prevalence and distribution of parasites in any given area is of fundamental importance. This subject appealed to Hall quite early in his professional career, and in 1912 he published an extensive report of the then existing knowledge of the distribution and importance of parasites of sheep and cattle in the United States. This paper was followed by a publication on the parasitic fauna of Colorado and by later papers of a similar character. Hall was an enthusiastic systematizer of knowledge and he felt that it was a considerable service to scientists, although to a less degree a contribution to science, to gather together under one cover scattered notes, papers and records, not readily available to parasitologists working in places where library facilities were inadequate.

In addition to his miscellaneous publications which contained new data, Hall published a large series of papers designed to acquaint veterinarians and stockmen with parasites and parasitic diseases. His bulletins on parasites and parasitic diseases of dogs and sheep, respectively, were widely distributed by the Department of Agriculture in response to specific requests from veterinarians, stockmen and dog owners. His paper on parasites of goats is still the only publication on this subject

available in the United States, and his many articles on parasitology published in veterinary journals were potent factors in arousing the interest of the veterinary profession in parasitology as an important phase of veterinary science.

Hall's most important miscellaneous publication was a paper on arthropods as intermediate hosts of helminths, published by the Smithsonian Institution in 1929. Although this paper is a compilation, Hall expended much time and effort on this work and, in the opinion of the writers, the paper in question is a valuable contribution to helminthology and medical entomology.

Hall's more popular papers, which appeared in the Scientific Monthly and elsewhere, are excellent literary compositions. Not all of these papers, however, deal with parasitology. In these popular papers Hall expressed his opinions on various social, political and economic problems which were as close to his heart as the science of parasitology.

The work on the incidence of trichinae in man, carried out in the National Institute of Health, was Hall's final chapter in a series of papers which, when taken together, constitute an encyclopedic treatise on parasitology, begun in the summer of 1907, abruptly terminated in the spring of 1938. Hall's studies on trichinae in man stimulated considerable interest in trichinosis among medical men, and it is safe to predict that this renaissance of interest in human trichinosis for which Dr. Hall was largely responsible will result in a careful study by medical men in this country of trichinosis as a public health problem.—BENJAMIN SCHWARTZ AND PAUL D. HARWOOD, *Zoological Division, Bureau of Animal Industry, U. S. Dept. of Agriculture.*

FORMATION OF THE EGG SHELL IN *FASCIOLA HEPATICA* AS DEMONSTRATED BY HISTOLOGICAL METHODS*

PEDRO KOURÍ AND RALPH W. NAUSS

There appears to be no well-settled conclusion concerning the mode of formation of the egg shell in *Fasciola hepatica.* Schubmann (1905) accepted the views of many previous and contemporary authors as to the rôle of Mehlis' gland (cochlear or shell gland) in the process. Henneguy (1906) concluded instead that the yellowish refractile granules, which the vitelline cells contain and liberate in abundance, play the principal part in supplying shell-forming substance. Leuckart, according to Henneguy, accepted this view. Henneguy also noted the striking resemblance of the Mehlis' and prostate glands, and suggested the secretions of the two were similar in character and function. Goldschmidt (1909) thought the yellow granules first described by Henneguy, represented the only substance involved in shell formation and supported Henneguy's interpretation of Mehlis' gland function. Tyzzer (1918) assigned to the secretion of Mehlis' gland an important accessory rôle in the process. He found in *Collyriclum faba,* a trematode of sparrows, that the globules are liberated suddenly and almost completely from the vitelline cells when they reach the oötype, and pointed out that the conditions which induced these changes are localized in the region where the secretion of Mehlis' gland is discharged. He comments: "The presence of peculiar granules in the cytoplasm of these shell gland cells, and in their slender protoplasmic processes extending to the oötype, is strongly indicative of a secretion of specialized type rather than an inert one in which the ova float as proposed by Goldschmidt." Augustine (1929) refers to Tyzzer's work, and states that in trematodes "the shell (egg) is probably derived from the fusing together of liberated yolk granules, while the glands (Mehlis' gland) surrounding the oötype probably secrete a liquid in which the ova are suspended." He does not, however, seem to concur in Tyzzer's contention that the secretion of Mehlis' gland must contribute something essential to the process of shell formation. Brumpt (1936) in a foot-note referring to Henneguy's work (1906) remarks that trematodes like *Sanguinicola* and *Zoögonus,* which lay eggs normally, do not have cochlear (Mehlis' gland) cells.

One of us (Kourí), first stimulated by pathological material derived

Received for publication, April 2, 1937.

* Contribution from The Faculty of Medicine, University of Havana, and The Department of Public Health, Cornell University Medical College, New York City. A preliminary report of this research, made before the Sociedad Cubana de Biologia, February 19, 1936, was published in the Revista de Parasitologia, Clínica y Laboratorio, March–April issue, 1936.

from human cases of *Fasciola hepatica* infection, undertook by careful histological studies to determine the significance of the roundish, yellow, refractile granules found so abundantly in the vitellogenous cells of this trematode. Subsequently, a survey of the literature relative to the whole subject of egg shell formation in trematodes revealed the facts above briefly reviewed. Realizing the excellence of the histological material available and the dearth of adequate treatment of this subject in modern texts, it was thought desirable to present the results of these histological observations.

METHODS

The living *Fasciola,* recovered from the bile duct of a recently killed animal, is washed with a gush of running water for 5–10 minutes. Following this it is brought into warm water (37.5° C) for a similar period, and then flattened and squeezed between two glass slides which are tied together with thread. So prepared it is placed in 10% formalin or Zenker's fluid for 12 hours. When the slides are untied, one of them is lifted carefully so that the flattened parasite may remain sticking upon the other. It is then replaced in the same fixing fluid for another 12 hours. Subsequent steps follow the usual technic for paraffin embedding, beginning with washing the specimen in running water for at least 5 hours after Zenker, less after formalin fixation. Furthermore, we think it advisable to introduce the fixed preparation, before washing it, into a bath containing pure acetic acid for some minutes or into a 10% solution of this acid for several hours. Such a procedure appears to improve the quality of the sections. Dehydration is in the usual alcohol series, and clearing in xylol.

Generally *Fasciola* sticks fairly well to the slide, but it is advisable to detach it before the final dehydration with absolute alcohol. The parasite usually remains flattened until placed in xylol, when one of its surfaces may bulge. To again flatten it during embedding we use a spatula made from a glass rod, one end of which has been bent at a right angle, and flattened so as to have the same form as the parasite. This spatula is kept at the embedding temperature. Upon placing the *Fasciola* and some melted paraffin into the mould, the parasite is immediately flattened with the warm spatula, and the latter carefully withdrawn after a thin layer of paraffin has solidified to hold it in place; the block may then be completed by filling the mould.

Serial sections of a considerable number of these parasites, oriented and studied in various ways, have been made during the past several years by one of us (Kourí). Staining methods were the acid-hemalum-eosin of Mayer, van Giesen, and the phosphotungstic acid-hematoxylin of Mallory. From the beginning, the structure of the vitellogenous glands and their secretions attracted our attention.

OBSERVATIONS

A. Vitellogenous Glands

Histologically, the vitellogenous glands are tubuloacinous or racemose. Each gland acinus opens, by a small tubule, into a wider duct leading to one of four collecting canals, which together extend laterally throughout nearly the whole length of the parasite. These four canals are situated two on the right, and two on the left. On each side there is a short anterior and a long posterior canal. Each pair of anterior and posterior collecting canals unite a little behind a horizontal line which passes the posterior border of Mehlis' gland, thus forming two transverse vitelloducts leading to the midline. The point of union in the midline is situated just behind Mehlis' gland, where a dilatation, the vitelline ampulla, is formed. This cone-shaped structure with apex directed forward penetrates Mehlis' gland, in which there exists posteriorly (Fig. 3) a space corresponding to its conical shape. The apex of the vitelline ampulla is prolonged as a narrow duct, which we shall call median vitelloduct, through which must pass all produces of secretion coming from the very numerous acini of the vitellogenous glands. The median vitelloduct continues, deep in Mehlis' gland, as the uterine tube, which, until it takes on a muscular layer, does not differ at all from the median vitelloduct. In the region where this muscular layer begins, the narrow part of the uterine tube is called the oötype. It shortly loses its muscular layer and widens out considerably (large lumen and thin wall) into the uterus proper.

By referring to fig. 3 and proceeding anteriad (from below upward) in the midline one observes the vitelline ampulla (vit. a.) opening into the vitelline duct at its apex, and then what is probably Laurer's canal (L. c.) near the middle of a sparsely cellular area that merges peripherally into a richly cellular zone. In the latter may be seen, on the left above, the first part of the uterine duct (u. d.). A little higher on the right is a section through the ovarian duct (ov. d.). Beyond, and thus anterior to Mehlis' gland, are three transverse sections of the oötype (oöt.). More to the left, pointing forward and upward, is a section passing through several convolutions of the first part of the uterus (u.).

The ovarian duct ends in a connection between the median vitelloduct and the oötype. We could not in our sections locate exactly the point where it ends. *Fasciola hepatica* has no seminal receptacle as an independent organ, but in the first portion of the uterus proper there are large areas containing accumulations of spermatozoa. These spermatozoa zones of the uterus probably correspond to the seminal receptacle occurring in other trematodes. Thus far, we have not been able to determine any duct with a proper wall which could be associated with Mehlis' gland. The zone of Mehlis' gland, however, is of importance, since it is where the different ducts of the female genital organs meet.

In summary, vitelline acini, tubules, ducts, collecting canals, transverse vitelloducts, vitelline ampulla, and the median vitelloduct constitute the continuous channel traversed by the vitellogenous cells from their origin in the acini to their arrival in the oötype and uterus. The ovarian duct leads to that part of the channel which passes from the median vitelloduct to the oötype. Beyond, in the first portion of the uterus, there are accumulations of spermatozoa. In this same first part of the uterus, outside and at a certain distance from Mehlis' gland, is the site of manufacture of the egg shell, chiefly and probably solely by the vitellogenous cells as we shall attempt to demonstrate histologically.

Fig. 1 is a low power ventro-dorsal photomicrograph of the anterior portion of *Fasciola hepatica* fixed flat in 10% formalin and stained with Mayer's acid-hemalum. Following the median line posteriad one notes the following structures:

(a) Oral sucker (o. s.) continuous with the pharyngeal bulb (ph. b.) posteriorly, both being of muscular type.

(b) The very short esophagus (e.) also continuous with the pharyngeal bulb anteriorly, bifurcating into two intestinal passages which in turn send out recurrent branches forward and outward, dividing and reaching the border of the parasite. These two principal trunks of the intestine form, in their beginning, an arch surrounding the cirrus pouch (c. p.) and the ventral sucker (v. s.). In the remaining parts of this parasite, the intestine is hidden behind the reproductive organs.

(c) Cirrus pouch (c. p.) with cirrus (c.), and the seminal vesicle (s. v.). In the clear space between the concavities of these structures is the metraterm or vagina (v.).

(d) Ventral sucker (v. s.) or acetabulum, in the midst of the last circumvolutions of the uterus.

(e) Sinuosities of the uterus (u.). The opacity and color of the contained eggs gives the black appearance.

(f) Mehlis' gland (M.), crossed from above downward and from left to right by the ovarian duct (ov. d.). Also the branched ovary (ov.), and transverse vitelloducts (t. v. d.), vitelline ampulla, with median vitelloduct at the posterior pole of Mehlis' gland.

(g) Anterior testis (a. t.) with its intricate ramifications, occupying more or less the middle third of the parasite. At the right the branches of the anterior testis pass beyond Mehlis' gland, and even reach the first sinuosities of the uterus. On the left they terminate lower down, appearing to blend with the posterior projections of the ovary, but without passing beyond, or communicating with it.

(h) Vitellogenous glands (vit. g.), embracing laterally the testes, ovary and uterus, which fill the central portions of most of the anterior two-thirds of the parasite. One can see the vitellogenous glands arising

laterally at the level of the anterior border of the acetabulum; their enormous number suggests a capability of providing the shell as well as food for the great quantity of eggs produced by *Fasciola.* On the other hand, it would seem improbable that so small an organ as Mehlis' gland could furnish the material required for making so many egg shells.

In fig. 2 is shown a section of the anterior third of *Fasciola hepatica* also under low power magnification, with most of the structures readily recognizable as described above for fig. 1. In the extreme lower mid-portion is Mehlis' gland (M.). It is shown again in fig. 4, where it is magnified about five times more. Its rounded shape with indented borders, its external layer of numerous peripheral cells, its clear central portion containing few cells, small disseminated nuclei and radiating filaments are noteworthy. In the lower part of fig. 4 the median vitelloduct (m. vit. d.) is very clear, filled with a mosaic of polygonal cells that have small nuclei. In the upper part, the ovarian duct (ov. d.) penetrates the margin of the gland. Nearby are three transverse sections of the first portion of the uterus (oötype), the central one containing a complete egg (shell, nutritive vitellus cells and ovum). In the center of the gland a small duct, probably Laurer's canal (L. c.), is seen sectioned obliquely. Filaments project inwards and outwards from the periphery of the gland establishing an intimate connection with the surrounding tissues. On the left, the clear canal filled with a dark substance is an intestinal trunk containing partially digested bile.

Fig. 5 shows a selected small portion of Mehlis' gland under higher magnification than fig. 4. It contains in its periphery a great many cells with clear protoplasm (M. g. c.) which stain very poorly. They present diffuse outlines with projections that anastomose with those of neighboring cells to form a network. Their clear, round nuclei, which contain little chromatin, show one or two indistinct nucleoli. In the left part of the field is a portion of the uterine duct (u. d.), full of vitellogenous cells (vit. c.).

In the hemalum-eosin stained sections, the tubulous acini, or vitello-acini (fig. 6), of the vitellogenous glands appear, more or less elongate according to the plane of cutting. Larger acini were 55–120 μ, with the differences due perhaps in part to orientation in the sections. They consist essentially of a thin layer of tissue forming their walls, with the granular, so-called vitellogenous cells (vit. c.) in the lumen. The number of vitellogenous cells in the vitello-acini varies according to the size of the acinus, one of large diameter showing seventeen. (We refer here to sections, and our descriptions apply only to elements seen in these sections.) These cells, originating in the vitello-acini, migrate through the various parts of the conducting system of the vitellogenous glands until they reach the first portion of the uterus, having traversed successively

during the latter part of their journey the vitelline ampulla, the median vitelloduct, and the oötype.

B. Vitellogenous Cells

These cells (figs. 5, 6, 7, 8, 9 and Plate VIII) originate in the gland acini, and upon reaching the first part of the uterine tube have two functions in connection with the formation of the egg of *Fasciola hepatica:* they supply building material for the egg shell, and they combine to form the food substance of the egg.

The vitellogenous cells in the acini and throughout the tubules, ducts, collecting canals, transverse vitelloduct, vitelline ampulla, median vitelloduct and oötype do not appear to undergo essential visible changes. They are shown best in figs. 6, 7, 15 and 16, and present a polygonal, frequently pentagonal form with rounded corners, sometimes almost circular. There is a clear, transparent, colorless protoplasm which usually contains an abundance of yellowish, refractile granules, similar in appearance to bile-stained fat droplets. When stained with hemalum-eosin, the granules, which may approach in size the cell nucleus, seem to adhere to the inner aspect of the cell membrane, and give the appearance of a cog-wheel with teeth on the inside circumference. The outlines of the cells are distinct, and appear to be reinforced by a yellowish refractile substance, similar to the granules mentioned. The nucleus is small, round, usually central, with a dense, dark, concentrated chromatin without definite nucleoli.

In size, the vitellogenous cells are relatively uniform. One large cell, 34 μ in greatest diameter, had a nucleus of 5.4 μ, granules to 3.5 μ. Forty granulations were counted in this cell. In another large cell of 28 μ diameter, the largest granule noted at 4.5 μ approached the diameter of the nucleus of 4.9 μ. Another 33×19 μ had a nucleus of 5 μ with largest granule of 4.3 μ. From a collecting duct cell diameters of 29 μ and 31 μ were noted.

C. Vitellogenous Cells upon Reaching the Uterus

When the vitellogenous cells reach the first portion of the uterus, they begin to undergo morphological changes, which become progressively manifest (figs. 8–10). The formerly clear, transparent and colorless protoplasm takes on a light violet color, due to the appearance of very fine granulations which react to the hemalum-eosin stain. The gross, yellowish, refractile granules are extruded, or expelled by the cells, so that they come to occupy the intercellular spaces. Likewise the yellowish, refractile substance, reinforcing the cell membrane, disappears. The nuclei do not undergo appreciable change but show more tendency toward eccentricity in position. The cells are diminished in size and now show considerable variations among themselves. Two, each 23 μ in diameter,

had nuclei respectively 4.6 μ and 7.8 μ in diameter. They are no longer regular polygons, but rather irregular or rounded in shape (figs. 8 and 9).

D. Formation of the Egg-Membrane

While the vitellogenous cells are undergoing the structural and morphological changes specified above, they appear to develop membranes (figs. 10 and 17). A number of the cells, varying in the sections from 6 to 20, according to the plane of section, form a group. Their yellowish, refractile granules, after being expelled or extruded, coalesce into larger masses. The substance of the latter completely envelops the cells which created them, forming a true membrane.

E. Formation of the Egg

The vitellogenous cells when grouped (figs. 10 and 11) receive in their midst an already fertilized ovum before the egg-membrane is completed. It will be recalled that the ovarian duct leads to a connection between the median vitelloduct and the oötype, the agglomerations of spermatozoa being found just beyond the latter in the first portion of the uterus. In this same part of the uterus, but beyond Mehlis' gland, the vitellogenous cells group to build their membranes. Although Mehlis' gland is in close proximity to the locus of formation of the egg shell, we have found no evidence that it is concerned directly with shell formation. As mentioned, its similarity in structure to the prostate gland (fig. 12) in *Fasciola hepatica* suggests quite a different function.

One may detect some structural differences between eggs recently formed in this proximal portion of the uterus, and those found distally, with various transitional stages.

F. Ripening of the Egg

The fertilized ovum, resulting from the fusion of a mature ovum and a spermatozoön, requires two additional materials, namely, vitellogenous cells and a protective egg-membrane. Ripening of the egg or ovum refers to transformations occurring from the time of its fertilization in the first part of the uterus to its expulsion from the trematode through the genital pore. The group of intra-uterine vitellogenous cells which produce yellow granules in the acini of vitellogenous glands supply also nutritive substances for the development of the future embryo. Absence of significant granules from cells which go to make up the vitellus is shown in figs. 11 and 18.

An unfertilized egg is one which is sterile because there has been no union of a spermatozoön with an ovum. An unripe egg is a normally fertilized ovum, with food substance and egg shell as it occurs in the first part of the uterine canal. Such unripe eggs have been found by one of us (Kourí) in the feces of patients with distomiasis who were being

treated with emetine. While this drug kills *Fasciola hepatica,* there also results a liberation of both ripe and unripe ova from the uterus of the disintegrating parasite. Such ova show signs of degeneration, possibly from the emetine which has already poisoned the parasite. Fertilization and ripening of the egg in *Fasciola hepatica* are independent of one another as is commonly observed biologically.

As the vitellogenous cells expel their membrane-forming granules, they decrease in size. Their protoplasm is more apt to take stains such as hemalum-eosin, owing to the apparent relative increase of their finer granulations. Thus the egg-membrane appears in sharper outline (fig. 11). When the egg has fully ripened, as in the last portions of the uterus and in the vagina, its covering-membrane is perfectly defined, with a darker yellow color inside. In the sections, one can count 40–50 vitellogenous cells, which are now much smaller, and have vividly stained protoplasm. Their diffuse outlines are easily confounded with those of neighboring cells, while the nuclei remain unchanged and easily recognized. In this stage, the vitellogenous cells present diameters of 12–16 μ, their nuclei about 5 μ.

Within every shell-covered egg, in the midst of its enclosed aggregation of vitellogenous cells (nutritive substance), there is generally but one ovum, in rare instances two. In sections, an ovum may not be observed within every egg shell, owing to the fact that the cut is not always at the proper level. When examining serial sections, however, every shell-covered egg will be found to contain an ovum. The ovum, as observed in the ovarian duct, is a regularly rounded cell (figs. 14 and 19), sometimes greater in length than width, having a diameter of 20–23 μ, protoplasm that stains well with eosin, and a large round nucleus, of 14–16 μ diameter. It contains a large, round, generally eccentric nucleolus, about 4 μ in diameter and a clear perinucleolar zone. The protoplasm of the ovum (fig. 14) takes the stain so intensely that it is sometimes difficult at first sight to distinguish the nuclear outline. When studying the ovum inside of the shell-covered egg, it is even more difficult to distinguish its structural features. It looks like a roundish, darkly-stained body of rather hyaline character. In iron-hematoxylin stained sections, one frequently observes chromosomes in mitosis, usually prophase or metaphase (fig. 20).

G. Mehlis' Gland

Reference has already been made to the striking morphological similarity of Mehlis' gland and the prostate gland (figs. 2, 4, 12, and 13) of *Fasciola hepatica.* This resemblance in appearance, structure and disposition of the cellular components of these two glands suggests the idea, as intimated by Henneguy (1906) and maintained by Goldschmidt

(1909), that both have a similar function. The location of each gland also appears significant. Mehlis' gland lies at a point where several female genital ducts (median vitelloduct, uterus, ovarian duct) converge and merge in the depth of the gland surrounding them (figs. 1, 2, 3 and 4). Through this narrow strait, fundamental materials that go to constitute the complete egg (*i.e.*, the vitellogenous cells, the ovum, and the spermatozoön which has to fertilize it) and the egg itself have to pass. The vitelline ampulla acts as a receptacle for vitellogenous cells and in the first part of the uterus the storage pouches of the spermatozoa are located.

The prostate gland (figs. 2 and 12) is likewise situated at a junction point, inside of the cirrus sac which surrounds also the seminal vesicle, ejaculatory duct, and cirrus. In the vicinity of the genital pore, the cirrus and the vagina (metraterm) terminate in close proximity to one another. These are narrow ducts through which spermatozoa and ova must pass. This being true, one suspects that Mehlis' gland, as well as the prostate gland, secretes some lubricant, which favors the forward movement of these genital products. However, we have not yet been able to satisfactorily demonstrate the similarity of function of these two glands histologically, in the way we have been able to prove, we believe, the membrane-forming function of the vitellogenous cells. If it is true that Mehlis' gland does not produce the egg-membrane, it ought to be deprived of every designation which suggests the idea that it has as its major function the formation of the egg shell. We should, therefore, always call it Mehlis' gland, and drop the designation shell gland, thus avoiding any discussion with regard to a questionable function.

DISCUSSION

In a preliminary study of the histology of *Clonorchis sinensis* we have found that its vitellogenous cells show morphological similarity to those of *Fasciola hepatica*. In both instances these cells abound in membrane-forming granules. It would seem reasonable to predict, therefore, that the granules perform similar functions in both species.

More extended investigations are desirable in order to ascertain whether the shell-forming function of vitellogenous glands is common among trematodes, and possibly among PLATYHELMINTHES generally.

SUMMARY

The vitellogenous cells of *Fasciola hepatica* are traced histologically from their locus of origin in the vitello-acini to the first part of the uterus where the ova are formed. These cells, which already show in the gland acini the presence of yellowish, refractile granules within the cytoplasm, carry these granules without appreciable change to the first

part of the uterus. Here ova may be observed in process of formation from groups of vitellogenous cells which divest themselves of their granules, each such group receiving a fertilized ovum into its midst. Our sections seem to show convincingly that the egg shell is evolved from the granules which the vitellogenous cells carry to the oötype. This is contrary to the more generally accepted view that Mehlis' gland supplies material out of which the egg shell of *Fasciola hepatica* is fashioned.

Attention is redirected to the striking histological similarities of Mehlis' gland and the prostate gland in *Fasciola hepatica* and to their anatomical relationships. It is believed that both glands have similar functions in supplying a lubricant to facilitate the ready passage of genital products such as vitellogenous cells, spermatozoa and ova through narrow ducts.

Bibliography

Augustine, D. L. 1929 In Hegner, R., Root, F. M., and Augustine. D. L. Animal Parasitology, with Special Reference to Man and Domesticated Animals. New York.

Brumpt, E. 1936 Précis de Parasitologie. Paris, 5th Ed. p. 551.

Goldschmidt, R. 1909 Eischale, Schalendruse und Dotterzellen der Trematoden. Zool. Anz. **34**: 481.

Henneguy, L. F. 1906 Recherche sur la mode de formation de l'oeuf ectolécithe du Distomum hepaticum. Arch. Anat. Micr. **9**: 47.

Schubmann, W. 1905 Über die Eibildung und Embryonalentwicklung von Fasciola hepatica L. (Distomum hepaticum Retz.) Zool. Jahrb. Jena **21**: 571.

Tyzzer, E. E. 1918 A monostome of the genus *Collyriclum* occurring in the European sparrow, with observations on the development of the ovum. J. Med. Research **38**: 267.

EXPLANATION OF PLATES

Abbreviations

a. t. ..... anterior testis
c. ..... cirrus
c. p. ..... cirrus pouch
cut. ..... cuticle
d. d. ..... deferent duct
e. ..... esophagus
ej. d. ..... ejaculatory duct
e. m. ..... enveloping membrane
i. ..... intestine
i. t. ..... intestinal trunk
L. c. ..... Laurer's canal
M. ..... Mehlis' gland
M. g. c. ..... Mehlis' gland cells
M. vit. d. ..... median vitelline duct
n. c. ..... nerve cell
oöt. ..... oötype
o. d. ..... oral sucker
ov. ..... ovary
ov. d. ..... ovarian duct
ph. b. ..... pharyngeal bulb
pr. g. ..... prostate gland
s. v. ..... seminal vesicle
t. vit. d. ..... transverse vitelline duct
u. ..... uterus
u. d. ..... uterine duct
u. w. ..... uterine wall
v. ..... vagina or metraterm
v. s. ..... ventral sucker or acetabulum
vit. a. ..... vitelline ampulla
vit. c. ..... vitellogenous cells
vit. g. ..... vitellogenous glands

PLATE I

FIG. 1. View of anterior third of *Fasciola hepatica.* Mayer's acid-hemalum stain.
FIG. 2. Section through anterior third of *F. hepatica.* Hematoxylin-eosin stain.

PLATE II

FIG. 3. Section of *F. hepatica* passing through region of Mehlis' gland.
FIG. 4. Another section showing Mehlis' gland on a larger scale than in Fig. 3.

PLATE III

FIG. 5. Cells of Mehlis' gland under high magnification.
FIG. 6. Section of acini of vitellogenous glands. Hemalum-eosin stain.

PLATE IV

FIG. 7. Section of a larger vitellogenous duct filled with vitellogenous cells.
FIG. 8. Section of part of uterus where the vitellogenous cells elaborate the egg shell.

PLATE V

FIG. 9. An enlarged portion of figure 8.
FIG. 10. Another portion of a section similar to Fig. 8, under a higher magnification.

PLATE VI

FIG. 11. Section of first portion of uterine tube showing a newly formed ovum. Hemalum-eosin stain.
FIG. 12. Section through cirrus pouch and prostate gland.

PLATE VII

FIG. 13. Cells of prostate gland under high magnification.
FIG. 14. Section of ovarian duct filled with ova.

PLATE VIII

FIG. 15. Vitellogenous cell from acinus of vitellaria.
FIG. 16. Vitellogenous cell from transverse vitelline duct.
FIG. 17. Vitellogenous cells in first part of uterus as they group themselves and excrete shell-forming granules which fuse to form the egg shell.
FIG. 18. Vitellogenous cells in mature egg.
FIG. 19. Ovum at terminus of oviduct.
FIG. 20. Ovum within egg undergoing mitosis.
FIG. 21. Cells of Mehlis' gland.

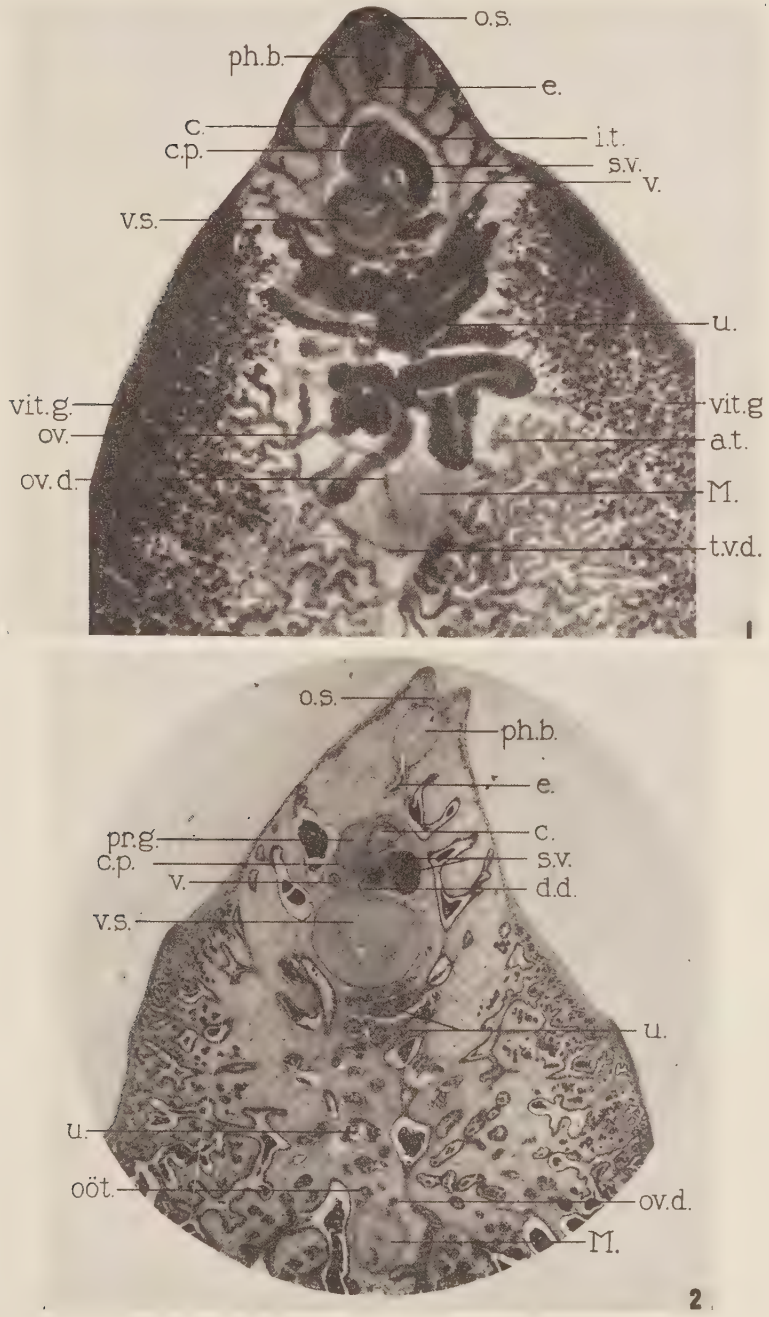
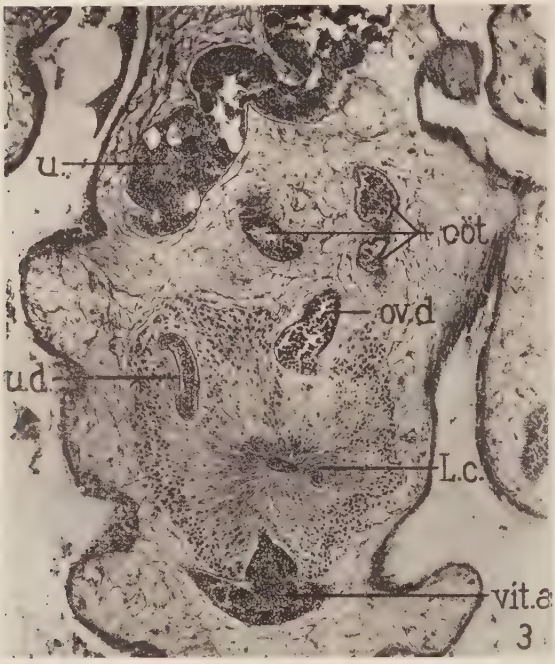

o.s.
ph.b.
e.
c.
c.p.
i.t.
s.v.
v.
v.s.
u.
vit.g.
ov.
ov.d.
vit.g
a.t.
M.
t.v.d.
1
o.s.
ph.b.
e.
pr.g.
c.
c.p.
s.v.
v.
d.d.
v.s.
u.
u.
oöt.
ov.d.
M.
2

PLATE I



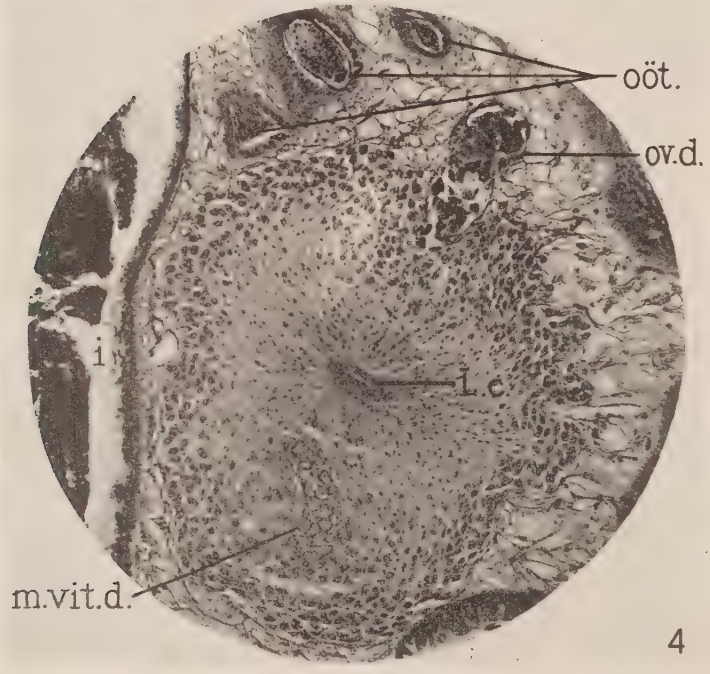
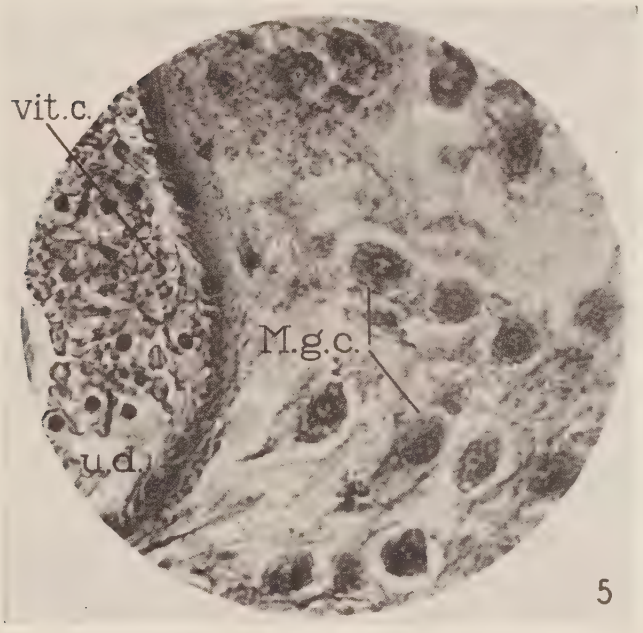


PLATE II



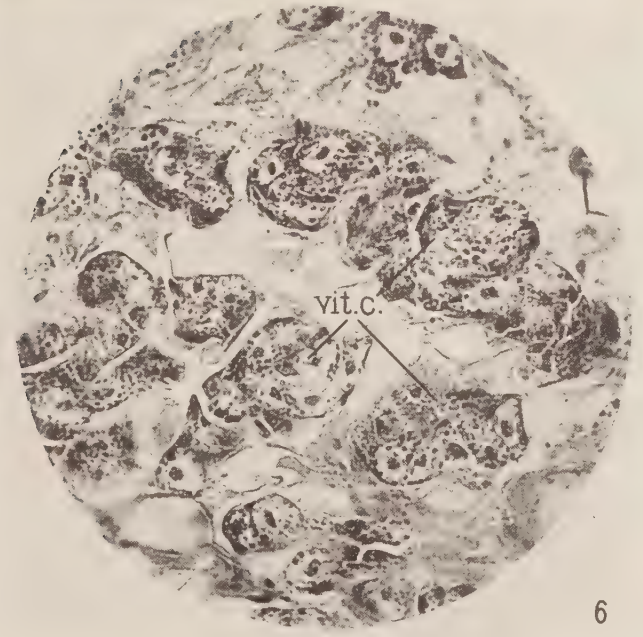


PLATE III

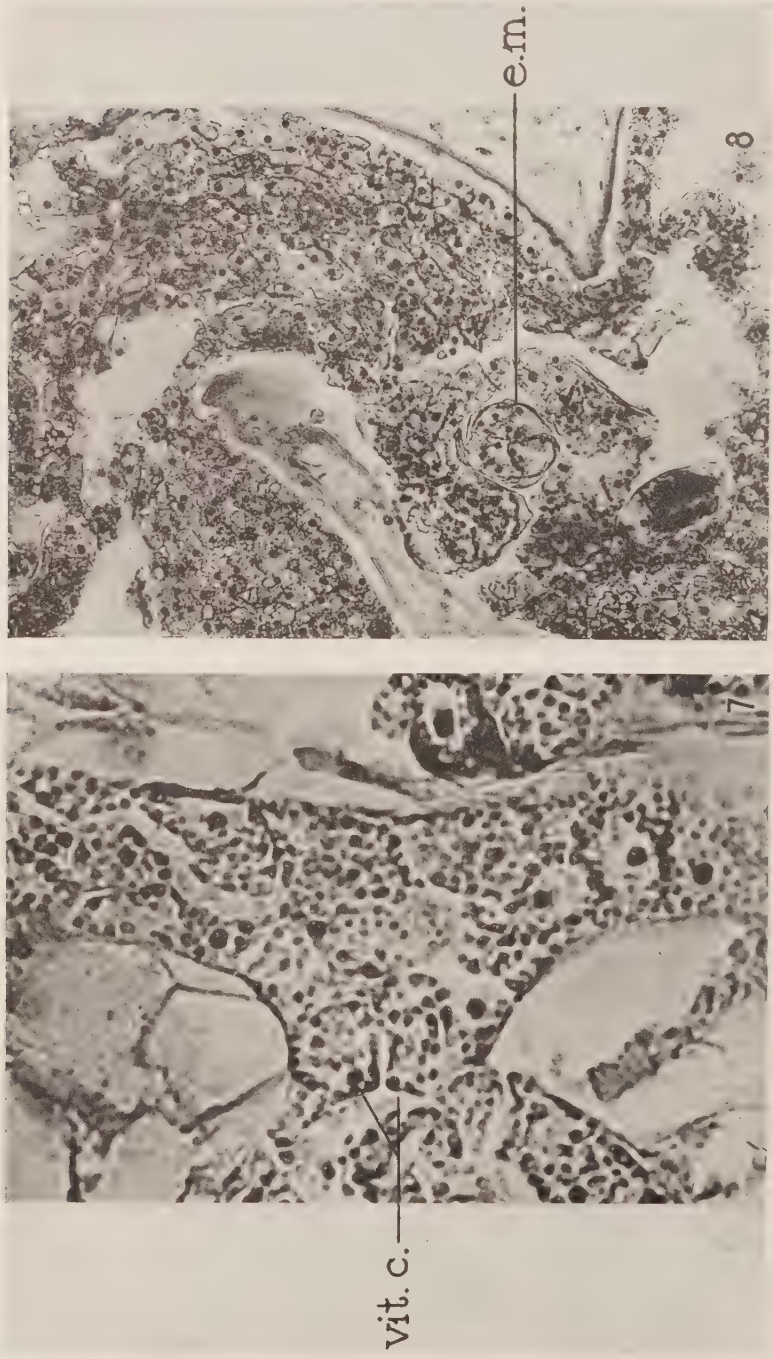


PLATE IV

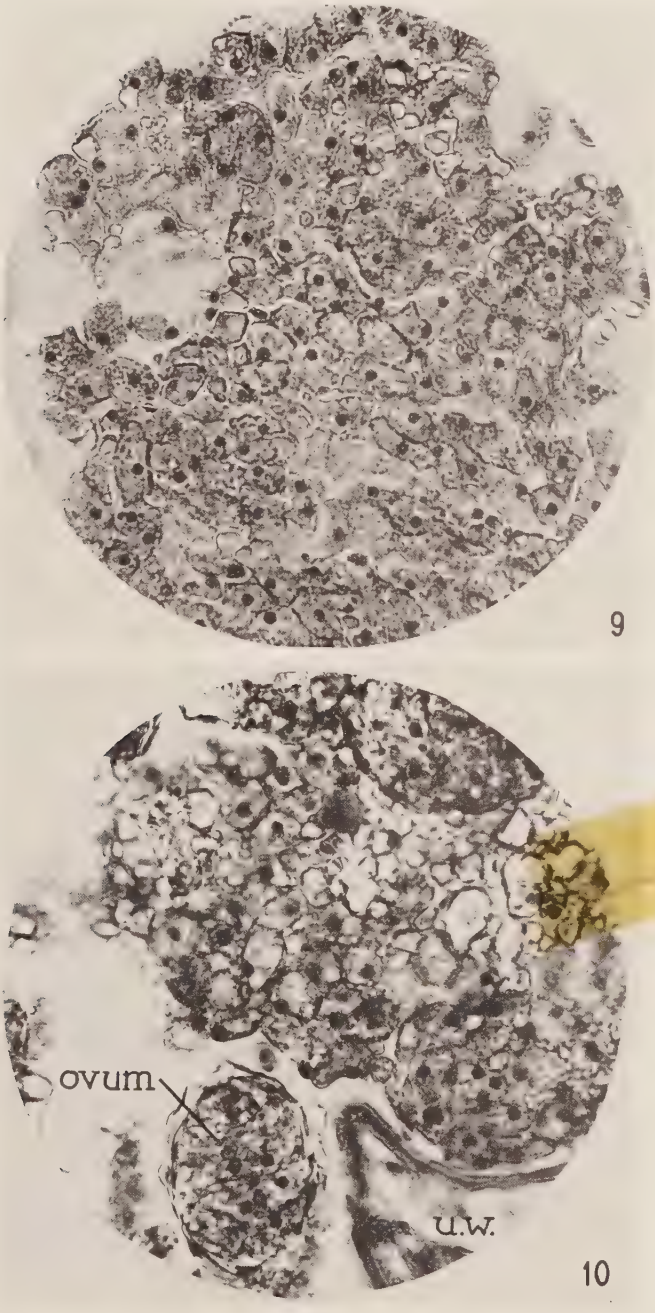
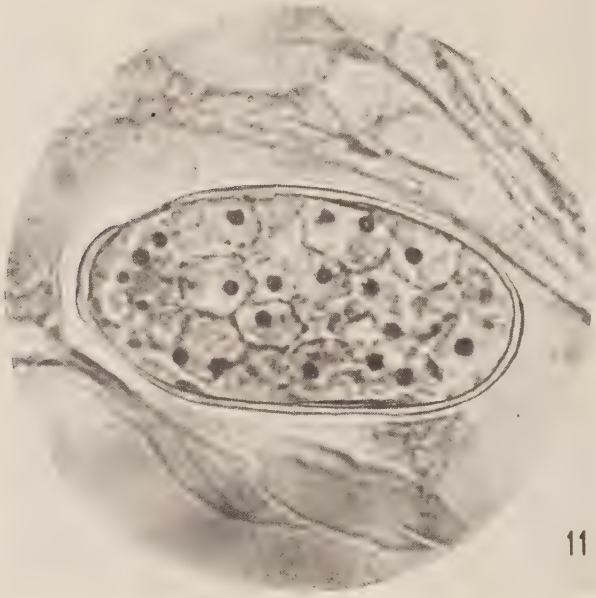


PLATE V



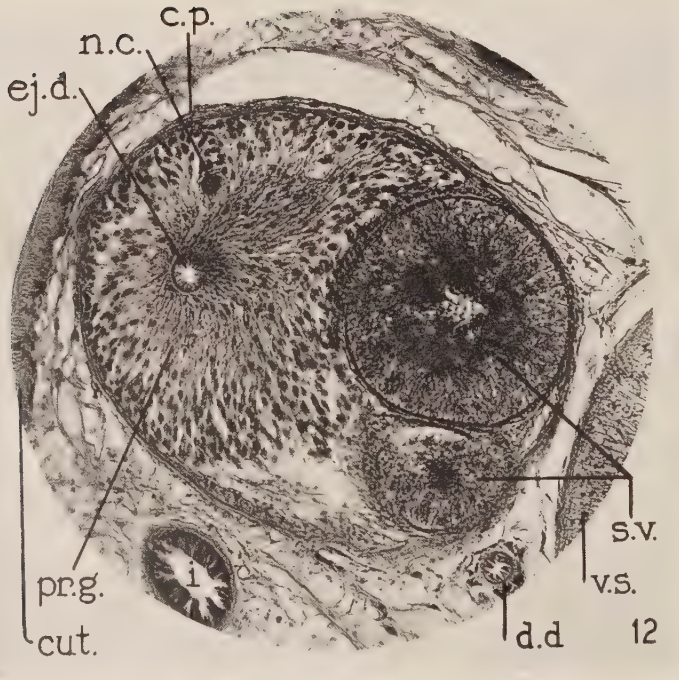
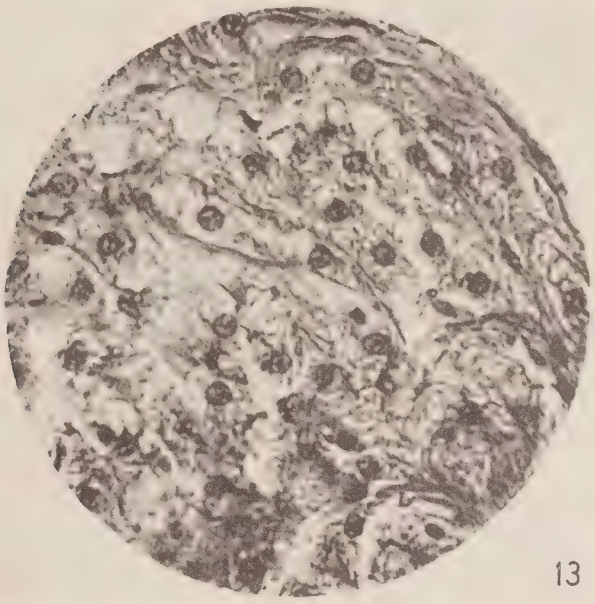


PLATE VI



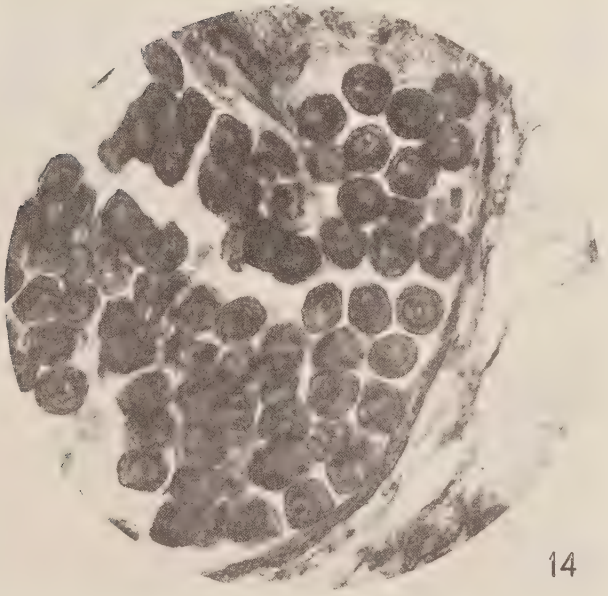
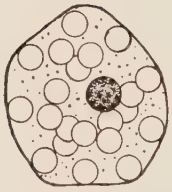
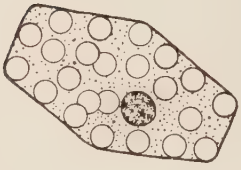


PLATE VII

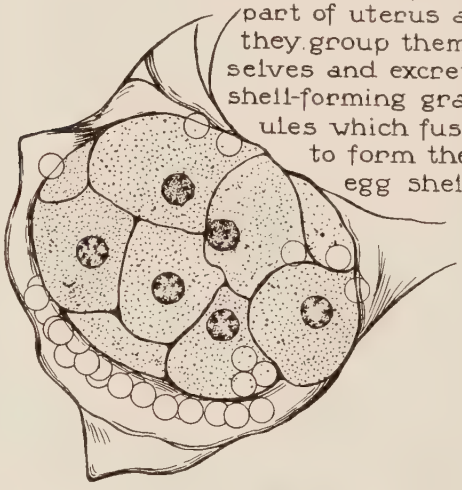


15. Vitellogenous cell from acinus of vitellaria.



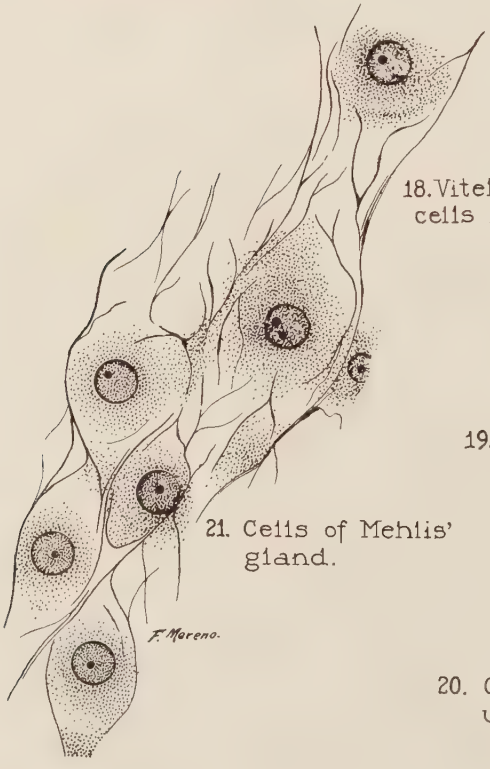
16. Vitellogenous cell from transverse vitelline duct.

17. Vitellogenous cells in first part of uterus as they group themselves and excrete shell-forming granules which fuse to form the egg shell.



18. Vitellogenous cells in mature egg.

19. Ovum at terminus of oviduct.



21. Cells of Mehlis' gland.

20. Ovum within egg undergoing mitosis.

PLATE VIII

THE REACTION OF THE IMMUNE INTESTINAL EPITHELIUM OF THE RAT TO REINFECTION WITH *EIMERIA NIESCHULZI*

NEAL F. MOREHOUSE[1]
Department of Zoology and Entomology, Iowa State College[2]

Hall and Wigdor (1918) were apparently the first investigators to report the successful immunization of an animal (dog) to coccidia. Andrews (1926) confirmed their results when he reported immunity in dogs and cats which lasted for at least seven months. He reported (1930), however, that dogs and cats kept in the laboratory for a year or more following immunization, showed evidence of repeated attacks of coccidiosis with intervals of apparent freedom from disease. He states in this regard, "Whether these repeated infections are newly acquired or whether they are the relapse of a latent infection, as in the case of bird malaria is not known." If the relapse were due to latent infection, coccidia could no longer be considered to have a self-limited life cycle. Furthermore, it appears that relapse could be produced by reducing the animals' resistance to disease. Further experiments by Beach and Corl (1925), Johnson (1927), Tyzzer (1929), Tyzzer, Theiler and Jones (1932), Becker and Hall (1932) and others show conclusively that a certain degree of immunity can be established against most, if not all, species of coccidia. The mechanism whereby the immune host resists infection, however, remains questionable. Tyzzer, Theiler and Jones (1932) explained the disappearance of *Eimeria necatrix* Johnson (1930) from the tissues of immune chickens as a destruction of sporozoites due to a failure of the invaded cells to react favorably toward the growth of the parasite. Becker (1934), however, was unable to detect developing stages of coccidia in the tissues of immune rats which had subsequently received heavy doses of *Eimeria miyairii* = *E. nieschulzi* (see Roudabush, 1937).

The following problems are suggested by the review of literature just presented. (1) Does the coccidium, *Eimeria nieschulzi,* have a truly self-limited life cycle? Or, is the infection carried along indefinitely in a latent condition such as occurs in birds parasitized by malaria and in sparrows infected with *Isospora?* (2) Does any intracellular development of *E. nieschulzi* occur in immunized rats when viable sporulated oocysts are ingested?

Received for publication, October 25, 1937.

[1] The author extends his thanks to Dr. E. R. Becker for his suggestions in outlining the problem and his constant guidance and helpful criticism concerning this study.

[2] This study was made under Industrial Science Project No. 40.

METHODS

The albino rats used throughout these experiments were immunized by feeding with a stomach tube, three or more sub-lethal doses of the coccidium, *E. nieschulzi* Dieben (1924). When oocysts were no longer produced as a result of the infection, the animals were considered immune.

Fecal examination was accomplished by simple fecal smears and by modifications of the direct centrifugal flotation method used by Lane (1922) for diagnosis of hookworm infection. The procedure in the flotation methods was to select a few fecal pellets at random from the collecting pans beneath the wire bottom cages in which the rats were confined. These pellets were placed in a water tumbler containing 25 cc of water. After thorough comminution with an electric mixer, the content of the tumbler was equally divided between two 15 cc centrifuge tubes and was centrifuged at 800 r.p.m. for three or four minutes. The supernatant fluid was decanted and the contents of both tubes were combined into one. After adding water, the feces was brought into suspension by vigorous shaking and the tube was centrifuged as before. The supernatant fluid was replaced by a saturated sodium chloride solution. The feces was again brought into suspension and centrifuged for one minute at 1000 r.p.m. A portion of the upper layer was transferred to a microscope slide by means of a glass rod having one end flattened and enlarged to a diameter slightly less than that of the centrifuge tube. The sample, covered by a 2 mm coverslip, was thoroughly examined under the microscope. As a check on the accuracy of this technique, frequently a few oocysts from a stock culture were mixed with samples which had proven negative and were centrifuged. Upon examination, oocysts were detected in every case. The centrifugal flotation technique, further modified by the use of a solution consisting of 4 pounds of sugar and 3 pounds of water preserved with a crystal of thymol, was found effective in demonstrating small numbers of oocysts in a fecal sample. Most of the bubbles formed in the salt solution were eliminated when sugar was used, thus facilitating examination under the microscope. This method, hereafter referred to as the sugar flotation method, was used in experiments 2 and 3.

EXPERIMENTS

Experiment 1. An attempt to demonstrate latent infection in immune rats. Two male rats which had been immunized to *Eimeria nieschulzi,* as shown by negative fecal smears on the 8th, 9th and 10th days, following the last infective dose, were examined twice weekly from January 1 to March 1, 1935. All smears were negative for oocysts. On March 26, the rats were placed in individual cages and daily examination of the feces by the modified direct centrifugal salt flotation method was begun.

No oocysts were found in samples observed during the 24 day period of examination. At this time, infective doses of 4500 oocysts per rat produced a slight infection in one rat while the other remained negative.

Experiment 2. A second attempt to demonstrate latent infection in immune rats. Three rats which had been immunized to *E. nieschulzi* were placed in wire-bottom cages over collecting pans containing a 0.5% solution of cresol. In order to exclude any possibility of mechanical transmission by flies or other insects, a cover constructed of window screen was inverted over the cage. This proved an effective barrier since the bottom of the cover rested tightly against the concrete floor. Sugar flotations of samples selected at random from the accumulated excrement were examined every 3rd or 4th day. No oocysts were detected during the 54 day period of examination. The rats, however, were all slightly susceptible to an infective dose administered on the 54th day of the experiment.

Experiment 3. An attempt to produce a relapse in immune rats. It has long been known that animals in poor physical condition are more susceptible to diseases than healthy ones. Parturition is also thought to predispose the body to disease. Although no published statement has been found regarding the effect upon a coccidial infection, of the weakened condition caused by parturition, it has been suspected, by some investigators, of producing a relapse in animals which have recovered from immunizing doses of coccidia. The following experiment is an attempt to produce such a relapse.

One male and two female rats were thoroughly immunized to *Eimeria nieschulzi* and were placed together in a cage. As soon as the females became pregnant the male rat was killed. Sugar flotation examinations were made daily for 15 days, every other day for 10 days and on every 3rd or 4th day thereafter until the end of the experiment. Each of the 28 flotations were negative for oocysts. The 53 day period of examination covered the entire gestation period of both rats and 22 and 23 days respectively following parturition. After the regular examinations were discontinued, an attempt to reinfect the rats with 150,000 oocysts each was unsuccessful. These negative results show that the animals had lost none of their immunity during the course of the experiment.

Experiment 4. Does Eimeria nieschulzi undergo any intra-cellular development in immune rats? In order to determine whether any degree of development of intracellular stages of *E. nieschulzi* occurs in immune rats, it was necessary to examine a series of immunized animals which had received large infective doses of these organisms at varying intervals from the time of killing. It was assumed that, in case sporozoites enter the cells of immunized rats at all, the larger the infective dose of the coccidia the greater would be the chance of finding them in the tissues. There-

fore, the size of the dose was governed only by the quantity of oocysts at hand when the animals were infected. The approximate number of oocysts given to each rat was as follows: No. 1: 3,500,000; No. 2: 2,900,000; No. 3: 2,500,000; No. 4: 3,500,000; No. 5: 2,900,000; No. 6: 2,500,000; No. 7: 3,500,000; No. 8: 4,000,000; No. 9: 4,000,000; No. 10: 2,500,000; No. 11: 3,000,000; No. 12: 2,500,000. The interval between infection and killing was $12\frac{1}{2}$, 16, 17, 18, 19, $23\frac{1}{4}$, 24, 26, $30\frac{1}{4}$, 33, $37\frac{3}{4}$, and 41 hours respectively. Pieces of tissue taken from the center of the small intestine were fixed in Zenker's fluid for 24 hours and were embedded in paraffin. Sections 10, 7 and 5 microns thick were stained with methylene blue and eosin and mounted in balsam.

As a control, one susceptible rat was infected with 2,000,000 sporulated oocysts and killed 38 hours later. A piece of tissue taken from the center of the small intestine was treated in the same manner as those in the immune series. A study of the intestinal cells of this rat was made in order to determine the relative number of sporozoites or other developmental stages present in a susceptible infected rat. A total of 57 sporozoites were found on 18 sections 10 microns thick, or an average of 3.1 per section. No section contained less than one or more than seven although the presence of a large number of first generation schizonts showed that many more had entered the tissues.

Sporozoites of *E. nieschulzi* are discovered in the tissues with difficulty and only after much practice. Therefore, examination of slides from the immunized series of 12 rats was begun only after several weeks experience was obtained in distinguishing developmental stages of coccidia from various tissue elements of the rat. Five sections from each of the 12 rats were examined under an oil immersion lens. This number, judging by the sporozoite population in the control rat, is believed sufficient for drawing conclusions in regard to the presence or absence of developmental stages of coccidia.

With the exception of two rats, no developmental stages were found in the immune series. These exceptions were one sporozoite found in rat No. 9 and one first generation schizont found in rat No. 11. Both of these organisms appeared to be normal. Examination of additional material from the same rats failed to disclose the presence of other coccidia. It is believed that the presence of these two forms was due to the failure of the total intestinal surface of the animals to become thoroughly immunized. Although oocysts were not detected in the droppings of these rats following the previous test inoculations with sporulated oocysts, it is probable that some cells remained susceptible. Even though a sporozoite from a test inoculation had entered a cell and subsequently produced merozoites, the failure of the latter to find suitable cells would have halted development and thus have prevented the formation of oocysts. Although

this explanation seems to fit in with the facts demonstrated by this experiment, it is evident that there is need for further investigation in this respect.

DISCUSSION

Because of the regularity with which a coccidial infection develops and subsides, it is the opinion of many investigators that the life cycle is strictly a limited one. Nevertheless, there has been some difference of opinion and those who agree that it is strictly limited often disagree in regard to whether it is host-limited or self-limited.

Roudabush (1935) took merozoites of *Eimeria nieschulzi* on the 5th day of infection and injected them, by means of a stomach tube, into the intestine of a previously uninfected rat. Oocysts were found in the feces two days later, on the same day on which they would have been found in the original host. This experiment, although showing that under certain conditions the life cycle is a self-limited one, does not preclude the possibility of a latent asexual cycle. It is quite conceivable that some unfavorable environmental factor such as a defense reaction of the host (*e.g.* the production of immunity) might suppress the tendency toward the production of oocysts but still allow the infection to be carried on in a latent asexual condition. If this should occur, then we would expect the endogenous cycle to be carried to completion whenever these conditions were removed, that is, whenever the animal was again capable of producing oocysts.

It was shown in Experiment 1 that one of the immune rats which eliminated no oocysts during a period of 108 days was slightly susceptible to reinfection. This was also true of three rats in Experiment 2 which were free from oocysts for 54 days. It is believed that a latent infection, if one had been present in any of these four rats, would have resulted in the appearance of oocysts in the feces. The third attempt to determine whether a latent asexual cycle occurs in immunized rats was based on the assumption that gestation and parturition bring about a weakened condition in the mother and thus predispose her to disease. Thus, a rat, if it harbors a latent infection, might be expected to produce oocysts while in this weakened condition. The fact that no oocysts were detected is considered as additional evidence against the existence of latent infections in rats.

In view of the results obtained in Experiment 4 it appears that sporozoites although they were found free in the intestinal lumen of the immune rat $12\frac{1}{2}$ to $30\frac{1}{4}$ hours after reinfection, do not normally undergo intracellular development. If sporozoites should enter the intestinal cells of immune rats unhindered, then a number comparable to that found in the susceptible rat should be present at some time following the reinfection. Rats were killed and examined at approximately 2 to 4 hour intervals

from 12½ to 41 hours following infection. Only one sporozoite and one first generation schizont could be found in 60 cross sections on slides of intestine prepared from these animals, while an average of 3.1 sporozoites and many schizonts were found on each section from the control.

The schizont found in rat No. 11 was apparently undergoing normal development but the sporozoite found in rat No. 9 had undergone neither development nor degeneration as its shape was characteristic and the eosinophilic globules and nucleus were visible. Since no cell wall could be distinguished between the sporozoite and the lumen of the intestinal gland, there is some doubt that its position was intracellular. The only explanation that seems reasonable for the presence of these two forms is that immunizing doses of the parasite were not sufficient to confer complete immunity on their hosts. This explanation, however, is not to be interpreted as a statement that complete immunity is an impossibility, for the remaining ten rats in this experiment gave evidence to the contrary.

It is of interest to note that the theory of immunity in coccidiosis which supposes a sensitization of the host cells as providing an unfavorable environment for the development of sporozoites does not hold true in rats infected with *Eimeria nieschulzi,* first, because sporozoites did not normally enter the intestinal cells of immunized rats, and second, because no evidence of degeneration was found in the two cases where the parasite had entered. Although the results of this experiment do not correspond, in detail, to those of Tyzzer, Theiler and Jones (1932) they are not necessarily contradictory since those investigators worked with a different species of parasite in a different host.

CONCLUSIONS

(1) Immunized rats were found not to harbor a latent infection of *Eimeria nieschulzi.*

(2) Intracellular development of *E. nieschulzi* did not normally take place in rats which had been specifically immunized against this parasite.

(3) The sensitization of epithelial cells, providing an unfavorable environment for the parasite, is not a factor in the immunity of rats to the coccidium, *E. nieschulzi.*

(4) Failure to find sporozoites within the epithelial cells of immunized rats, after heavy infections, indicates that immunity is the result of blocking of the entrance of sporozoites rather than an unfavorable environment within the cell.

BIBLIOGRAPHY

ANDREWS, J. M. 1926 Coccidiosis in mammals. Am. J. Hyg. **6**: 784–798.

————1930 Coccidiosis in birds and mammals. In, Problems and Methods of Research in Protozoology, by R. W. Hegner and J. M. Andrews. Chap. 31, pp. 281–302.

BEACH, J. R. AND CORL, J. C. 1925 Studies in the control of avian coccidiosis. Poultry Sc. **4**: 83–93.

BECKER, E. R. 1934 Coccidia and Coccidiosis of Domesticated, Game and Laboratory Animals and of Man. Ames, Iowa. Collegiate Press. p. 12.

BECKER, E. R. AND HALL, P. R. 1932 Quantitative, biometric and host-parasite studies on *Eimeria miyairii* and *Eimeria separata* in rats. Iowa State Coll. J. Sc. **6**: 299–316.

HALL, M. C. AND WIGDOR, M. 1918 Canine coccidiosis, with a note regarding other protozoan parasites from the dog. J. Am. Vet. Med. Assn. **53**: 64–76.

JOHNSON, W. T. 1927 Immunity or resistance of the chicken to coccidial infection. Oregon Agric. Exper. Sta. Bull. No. 230.

ROUDABUSH, R. L. 1935 Merozoite infection in coccidiosis. J. Parasitol. **21**: 453–454.

———1937 The endogenous phases of the life cycles of *Eimeria nieschulzi*, *Eimeria separata*, and *Eimeria miyairii*, coccidian parasites of the rat. Iowa State Coll. J. Sc. **11**: 135–163.

TYZZER, E. E. 1929 Coccidiosis in gallinaceous birds. Am. J. Hyg. **10**: 269–383.

TYZZER, E. E., THEILER, H. AND JONES, E. E. 1932 Coccidiosis in gallinaceous birds. II. A comparative study of species of Eimeria of the chicken. Am. J. Hyg. **15**: 319–393.

SPINITECTUS CORTI N. SP. (NEMATODA: SPIRURIDAE)*

V. N. Moorthy
Mysore State Department of Health, Bangalore, India

A few specimens of *Spinitectus* were obtained from the intestines of the fish, *O. gachua,* collected from the fresh-water ponds in the Chitaldrug District, Mysore State, India. This nematode appears to be a new species and for it the name *Spinitectus corti* is proposed in honor of Doctor W. W. Cort, to whom the writer is greatly indebted for the help and encouragement given.

Spinitectus corti N. SP.

(Figs. 1–4)

Description. Body filiform, widest near middle and slightly attenuated towards both extremities; anterior end bluntly conical; posterior end acutely pointed in some specimens, less so in others. Head not distinctly marked off from body. Cuticular spinous rings conspicuous, beginning about 0.07 mm from anterior end; first two rows very close together compared to other rows; total number of spinous rows varying from 45 to 51: anterior-most row contains 22 spines in a circle as observed in lateral view while in other rows the number varies from about 6 near tail to about 30 in widest portion of body; in anterior half of body spines arranged in regular circles, more posteriorly in semicircles, and in tail portion irregularly placed (fig. 1).

Female. 1.4 to 1.9 mm long by 0.14 to 0.18 mm in maximum width at middle of body. Stoma short, 0.035 to 0.040 mm long, funnel shaped. Oral opening terminal, guarded by three lips. At anterior extremity amphid and a pair of cephalic papillae present (fig. 4). Pharynx, tubular, distinct and about one-third as wide as muscular portion of esophagus. Esophagus with distinct demarcation between anterior muscular portion and posterior glandular portion; anterior muscular portion 0.13 to 0.14 mm long; posterior glandular portion, multinucleated, 0.28 to 0.37 mm long (fig. 4): esophago-intestinal valve distinct. Nerve ring 0.12 to 0.14 mm from cephalic extremity. Excretory pore at level of fourth row of spines (fig. 1). Intestine consisting of large number of cells containing yellowish brown granules and globules, and extending from about level of 10th row to 35th row of spines. Vulva situated in posterior third of

Received for publication November 2, 1937.

* Contribution from the Department of Helminthology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.

Acknowledgment is made to the International Health Division of the Rockefeller Foundation for having granted the fellowship which made this work possible. The writer is indebted to Dr. B. Schwartz, Chief of the Zoological Division, U. S. Bureau of Animal Industry, for the privileges of working in his laboratory, and to Dr. B. G. Chitwood for valuable help and guidance.

body, close to anus, 1.23 to 1.60 mm from cephalic extremity; vagina long, muscular, extending anteriorly about 0.18 mm from vulva, and terminating at bifurcation of uterus (fig. 1). Uteri filled with eggs, occupying greater part of interior of body and extending anteriorly as far as middle of glandular portion of esophagus. Anus near caudal extremity, about level of 40th row of spines. Tail 0.10 to 0.13 mm long; in some specimens, spear-shaped and acutely pointed at tip, devoid of spines in its posterior third (fig. 2), while in others, conoid in shape, blunt and rounded at tip and spines extending to tip (fig. 3). Phasmids slightly anterior to tip of tail (fig. 2). Mature eggs in uteri and vagina, 0.028 to 0.031 mm by 0.016 to 0.018 mm, ellipsoidal, with thick transparent shells and without polar filaments.

Male. Unknown.

Habitat. Intestine of *Ophicephalus gachua.*

Locality. Chitaldrug District, Mysore State, India.

Specimen. U. S. Nat. Mus. Helm. Coll. No. 9130.

This species may be differentiated from the other species of the genus by its short stoma; its small size, being the smallest of all known species; its relatively greater width compared to length; and in having the first two rows of spines comparatively closer together than the other rows.

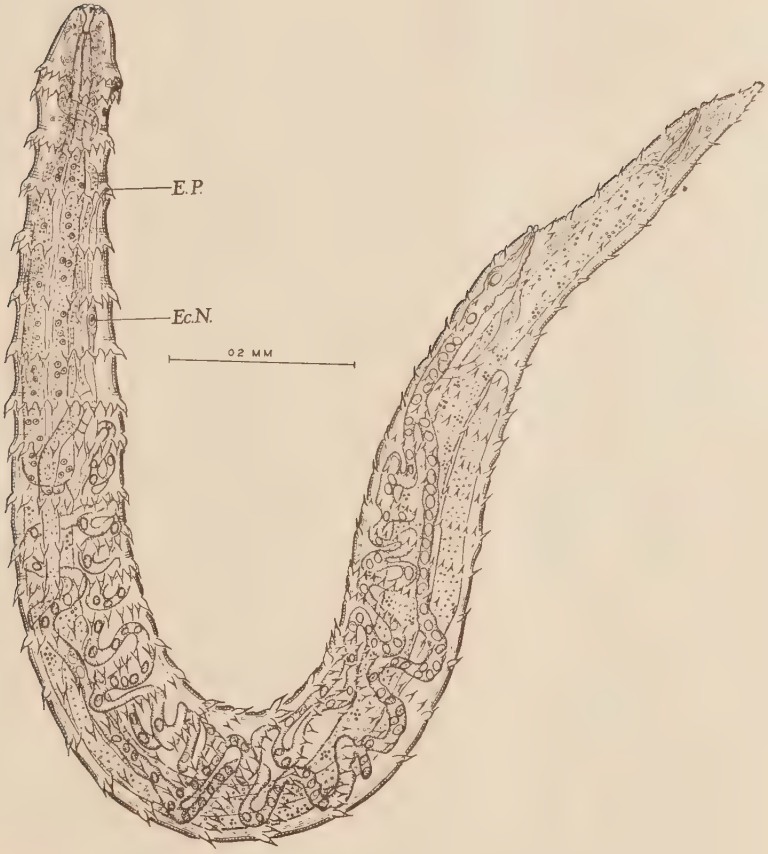


FIG. 1. *Spinitectus corti,* female, lateral view. E.P., excretory pore; Ec.N., excretory cell nucleus.

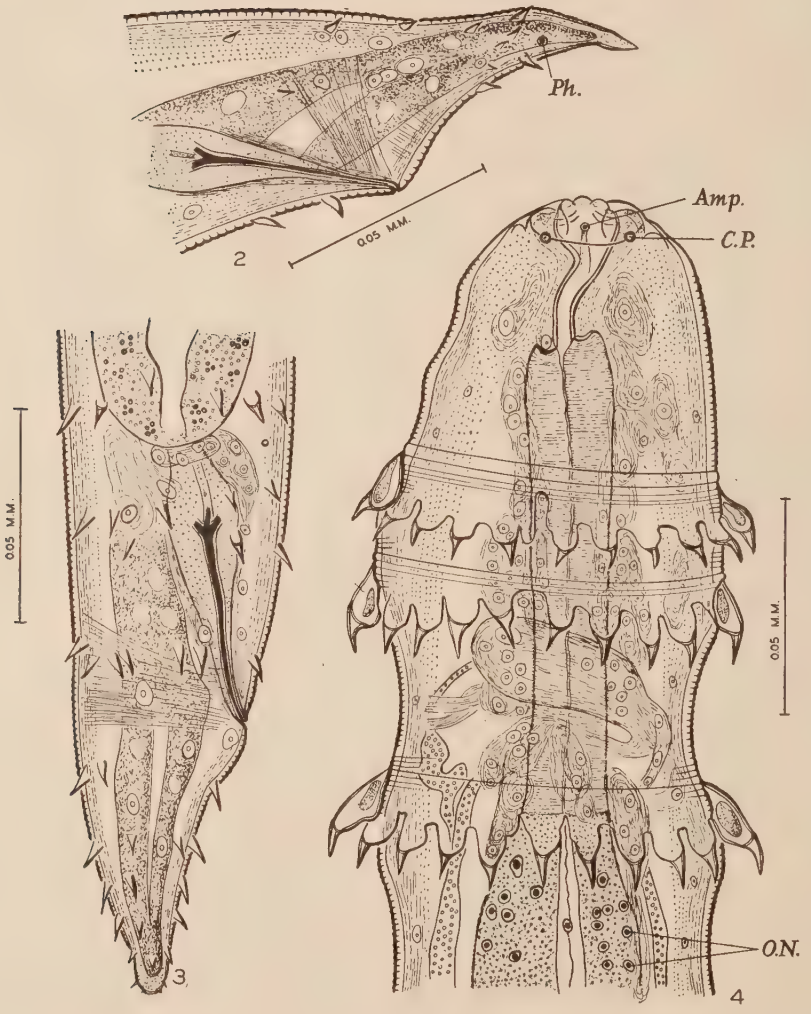


Fig. 2. Tail of female, with pointed posterior extremity; devoid of spines in the posterior region. Ph, phasmid.

Fig. 3. Tail of female, with rounded posterior extremity; spines extend to the tip of the tail.

Fig. 4. Female, esophageal region, lateral view. Amp., amphid; C.P., cervical papillae; O.N., esophageal nuclei.

OBSERVATIONS ON THE LIFE HISTORY OF *CAMALLANUS SWEETI**

V. N. Moorthy

Mysore State Department of Health, Bangalore, India

In a previous publication the writer (Moorthy, 1937b) gave a description of the adult of *Camallanus sweeti* and included a brief account of its life history, suggesting that this parasite requires the intervention of two intermediate hosts, cyclops and a smaller species of freshwater fish, before final development can take place in the definitive host, a freshwater fish, *Ophicephalus gachua*. The present paper records the general field and laboratory observations that have been made on the life history of the parasite. A brief description of developmental stages in the final and intermediate hosts is also given.

INCIDENCE OF INFECTION IN FISH

Of the five larger species of freshwater fish, *Mystus cavasius* (Ham), *Barbus sarana* (Ham), *Cirrhina fulungee* (Sykes), *Mastachembelus pancalus* (Ham), *Ophicephalus gachua* Ham, examined from different freshwater ponds in the Chitaldrug District, Mysore, India, only *O. gachua* was found to be infected (95 per cent) with adult *C. sweeti* under natural conditions. From the observations so far made this appears to be the only species of adult camallanid present in this fish.

FIRST INTERMEDIATE HOST

Nearly three thousand specimens of cyclops were examined from one of the freshwater ponds in which large numbers of infected *O. gachua* were present. Out of these only two were found infected, each with a single nematode larva. One of them (adult female *Mesocyclops leuckarti*) was infected with *Spiroxys* sp. and the other (adult female *Mesocyclops leuckarti* bearing ovisacs) with *Camallanus* sp. As mentioned subsequently, this larva differs structurally from the third stage larva obtained from cyclops infected in the laboratory with the first stage larva

Received for publication December 19, 1937.

* Contribution from the Department of Helminthology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.

Acknowledgment is made to the International Health Division of the Rockefeller Foundation for having granted the fellowship which made this work possible.

The writer is greatly indebted to Drs. W. W. Cort, E. W. Price, G. F. Otto, and P. Parthsarathy for the helpful criticism and advice given in the course of this work. To Dr. B. Schwartz, Chief of the Zoological Division, U. S. Bureau of Animal Industry, the writer's appreciation is due for the privileges of working in his laboratory, and to Dr. B. G. Chitwood the writer wishes to express his gratitude for valuable help and guidance.

of *C. sweeti*. Further attempts to obtain specimens of cyclops infected with *Camallanus* larvae under natural conditions were unsuccessful. It seems curious that the infection of cyclops under natural conditions is so very scarce, though the infection of the definitive host under natural conditions is as high as 95 per cent. In the case of *Dracunculus* infection also, although 50–60 per cent of the people using a particular source of drinking water became infected with dracontiasis, attempts to obtain specimens of infected cyclops under natural conditions in water supplies had invariably proved unsuccessful until 1936. It was found out subsequently, however (Moorthy and Sweet, 1936), that infected cyclops could be obtained only when they were sought during the infective season when contamination of the water sources with fresh *Dracunculus* larvae was highest. This occurred when there were large numbers of very early blister stage cases of dracontiasis in the village. It is possible that in *Camallanus* infection, also, there is a particular period of the year when infection of cyclops is at its maximum, and it is probably only during this period that one would succeed in getting the infected cyclops. Since *O. gachua* normally lives in freshwater ponds containing large volumes of water, unless a large percentage of cyclops are infected it would be difficult to catch them in the usual representative collections made. It has been further observed in the laboratory that in the case of *C. sweeti* infections it is only the females of *M. leuckarti* bearing ovisacs that become infected easily. If this should be true under natural conditions also, then these specimens of infected cyclops, due to the weight of the larvae and the ovisacs, will have a tendency to remain at the bottom of the well. Consequently in wells containing large volumes of water, when using usual methods of collecting cyclops by means of a tow net, these infected specimens are possibly missed.

In the majority of freshwater ponds in Chitaldrug District, Mysore State, India, in addition to *O. gachua,* large numbers of the smaller species of cyclops-eating fish are also present. It appears likely that a large percentage of the infected cyclops are eaten by these smaller species of fish, as indicated, for instance, by a high incidence of infection of the third stage larva of *C. sweeti* (90 per cent) in *Lepidocephalichthys thermalis* (C.V.). This is probably another reason for the scarcity of infected cyclops under natural conditions.

M. leuckarti Claus and *M. hyalinus* Rehberg are the most common species of cyclops in the freshwater ponds of Chitaldrug District. Of these, adult female *M. leuckarti* bearing ovisacs appear to be more easily infected with *C. sweeti* under laboratory conditions than are those of *M. hyalinus*. In one experimental infection the following percentages of infection were observed: *M. leuckarti* bearing ovisacs 75 per cent; *M. leuckarti* (both mature and immature forms) 4 per cent; *M. hyalinus* (both mature and immature forms) 1 per cent.

The following observations have been made on the experimental infection of cyclops (*M. leuckarti* and *M. hyalinus*) with first stage larvae of *C. sweeti:*

1. Maximum percentage of infection was observed when large numbers of adult female *M. leuckarti* were placed in a small quantity of water containing fresh *Camallanus* larvae obtained from adult worms, the cyclops having been starved for 36 hours previous to infection by isolating them in distilled water.

2. When mature and immature forms of both species of cyclops are used in the experiment only 25 to 30 per cent of them become infected, the rest being resistant, no matter how long they may be kept in contact with the larvae.

3. The cyclops become infected by swallowing the larvae. Four to eight hours after exposure to infection the larvae reach the body cavity where they undergo the early developmental changes described later.

4. A single cyclops may contain 12 to 15 larvae. These heavily infected individuals were sluggish and had a tendency to remain at the bottom of the container.

5. The longest period during which infected cyclops could be kept alive in the laboratory was 52 days in hot weather (90 to 102° F) and 70 days in cooler weather (55 to 70° F).

6. Attempts to infect cyclops with the third stage larvae obtained from other infected cyclops were always unsuccessful.

7. When specimens of cyclops infected with *Camallanus* larvae are superinfected with the fresh larvae of *Dracunculus medinensis,* it was observed that the latter underwent their usual developmental changes but the time required for this to take place was prolonged by 5–8 days. In a few instances the larvae developed only as far as the first or second molt, further development being completely arrested, although the cyclops remained alive for over 4 weeks in the laboratory. When, however, cyclops infected first with *Dracunculus* larvae were superinfected with fresh *Camallanus* larvae, the *Camallanus* larvae, except in a few instances, underwent complete development in approximately the same time as in cyclops not infected with *Dracunculus* larvae.

8. Cyclops infected with either *Camallanus* or *Dracunculus* larvae do not seem to develop fresh ovisacs. Infection with either species of nematode larvae seems to affect in some way (by pressure atrophy?) normal functioning of the genital system of cyclops.

EFFECT OF FRESH BILE (UNDILUTED) OR HYDROCHLORIC ACID (0.1 TO 1.0 PER CENT) ON CYCLOPS INFECTED WITH *Camallanus* LARVAE

Undiluted fresh bile of *O. gachua* killed cyclops in about five minutes and activated the larva in identically the same manner as in cyclops in-

fected with *Dracunculus* larvae (Moorthy, 1935). The activated larva makes frantic efforts to break through the anterior and posterior antennae, legs and various segmental joints of the cephalothorax and abdomen. It is only when the larva reaches the caudal ramus or the anal segment that it succeeds, like the *Dracunculus* larva, in escaping through a hole made there apparently by the persistent cutting and screwing movements of its oral extremity. Activation of the larva was observed in 108 specimens of infected cyclops treated with bile, but in only three instances did larvae succeed in escaping through the anal segment. The time taken for the larvae to escape varied from 50 minutes to 2 hours. When larvae did not succeed in breaking through the exoskeleton they usually died in the body cavity of the cyclops after 3–4 hours of active motion.

Fresh bile from *Barbus* (*Puntius*) *ticto* (Ham) and *Barbus puckelli* Day had the same effect as that of *O. gachua:* the cyclops were killed in one to two minutes. Of 32 infected cyclops treated with these biles, escape of larvae through anal segment or caudal ramus took place in four instances, at intervals varying from 48 minutes to 3 hours.

Bile of goat, sheep, and dog killed cyclops in 25 to 30 minutes; the larvae were activated only slightly, and in none of 20 specimens of infected cyclops treated in this way did the larva succeed in escaping.

A solution of preserved ox bile in distilled water in strengths varying from 10 to 20 per cent had little or no effect on the infected cyclops. It did not kill them for nearly three hours, and the activation of the larvae was not observed in any of the 20 specimens treated with this solution.

Hydrochloric acid in strengths varying from 0.1 to 1.0 per cent killed the cyclops in two to three minutes, but the *Camallanus* larvae, unlike the *Dracunculus* larvae, were only slightly activated. Escape of larvae was not observed in any of 20 specimens of cyclops treated.

The stimulation of *Camallanus* larva to activity by fresh bile seems to be definite since it was observed in all the specimens studied. Actual escape of the larva appears to be by chance depending possibly on the degree of digestion and softening of the exoskeleton of cyclops effected by the bile. In the laboratory, of 140 infected cyclops treated with fresh fish bile the larvae escaped from only seven. In the fish, however, it is likely that this release of larvae takes place easily and freely by the complete breakdown of the exoskeleton of cyclops during the usual processes of digestion.

SECOND INTERMEDIATE HOST

In the course of certain other investigations on the "biological method of control of dracontiasis," the following four species of small cyclops-eating freshwater fish were found to contain in their intestines, under natural conditions, the third stage larvae of *C. sweeti: Barbus puckelli*

Day, 20 per cent; *Barbus* (*Puntius*) *ticto* (Ham), 15 per cent; *Lepidocephalichthys thermalis* (C.V.), 90 per cent, and *Gambusia* sp. (introduced from Italy), 5 per cent. The larvae found in *B. ticto* and *Gambusia* were structurally identical with the third stage larva of *C. sweeti* from experimentally infected cyclops. In *B. puckelli* and *L. thermalis,* in addition to the larval stage found in cyclops a few were found (3 and 42 per cent respectively) that had undergone certain further developmental changes to be described later.

DEFINITIVE HOST

In the final host, *O. gachua,* infected under natural conditions the following different developmental stages of *C. sweeti* were found in the intestines: first stage larvae (1 per cent); third stage larvae, in the same stage of development as in experimentally infected cyclops (3 per cent); late third stage larvae in the same stage of development as in *B. puckelli* and *L. thermalis* (10 per cent); late fourth stage larva (0.5 per cent); early fifth stage larva (0.5 per cent); mature adult females (92 per cent); mature adult males (99 per cent).

DESCRIPTION OF THE DEVELOPMENTAL STAGES OF *C. sweeti*

Specimens of first stage larvae were obtained from the mature *C. sweeti* taken from *O. gachua.* The different developmental stages in cyclops came from experimentally infected cyclops. The stages described from the second intermediate and final hosts were from fish infected under natural conditions. The measurements of the larvae in different developmental stages, are summarized in table 1. In table 2 de Man's proportional measurements are given for α = total length/body width, β = total length/esophagus length and γ = total length/tail length. The location of the genital primordium or vulva from the anterior extremity is given as a percentage of total body length. It is seen from the table that as the first stage larva grows to the adult (early fifth stage) the value of α and γ steadily increases, suggesting that increase in body length is proportionally greater than corresponding width of body or of length of tail. There is not, however, much variation in the value of β for different developmental stages, suggesting that increase in length of esophagus is in proportion to increase in body length in different developmental stages of *C. sweeti.* Description and measurements are from specimens fixed and preserved in 2 per cent formalin or fixed, stained, and preserved by the author's method (Moorthy, 1937a).

First stage larva (Plate I, figs. 1 and 2). Measurements given in tables 1 and 2 are the average of 10 specimens selected at random.

TABLE 1.—*Measurements of different developmental stages of* Camallanus sweeti

No.	Stage of development of larvae	Specimens examined	Specimens measured	Body measurements (in mm)									
				Total length	Greatest width	Stoma	Nerve ring (from anterior end)	Excretory cell or pore (from anterior end)	Anterior[1] esophagus	Posterior[2] esophagus	Genital primordium or vulva (from anterior end)	Anus (from anterior end)	Tail length
1	1st stage larva	large number	10	0.24	0.014	....	0.03	0.09 (EC)	0.04	0.02	0.14 (GP)	0.20	0.04
2	Late 1st stage larva, molting	1	1	0.26	0.014	....	0.03	0.09 (EC)	0.05	0.02	0.15 (GP)	0.22	0.04
3	Early 2nd stage larva	1	1	0.41	0.023	0.007	0.04	0.05 (EP)	0.05	0.07	0.23 (GP)	0.37	0.04
4	2nd stage larva, molting	10	3	0.42	0.031	0.015	0.04	0.05 (EP)	0.08	0.08	0.23 (GP)	0.39	0.03
6	3rd stage larva (from experimentally infected cyclops)	large number	10	0.56	0.035	0.034	0.08	0.08 (EP)	0.16	0.10	0.37 (GP)	0.52	0.04
7	Developed 3rd stage larva in 2nd intermediate host (natural infection)	large number	4	0.74	0.034	0.035	0.08	0.08 (EP)	0.17	0.14	0.46 (GP)	0.70	0.04
9	Late 4th stage larva, molting (natural infection from intestine final host)	1	1	1.25	0.046	0.035	0.09	0.11 (EP)	0.20	0.20	0.70 (V)	1.20	0.06
10	Early 5th stage larva (natural infection from intestine final host)	1	1	1.98	0.060	0.046	0.10	0.12 (EP)	0.24	0.28	1.17 (V)	1.93	0.06

[1] Anterior portion of esophagus, from anterior end to commencement of glandular portion.
[2] Posterior portion of esophagus, from commencement of glandular portion to esophago-intestinal valve.
EC = excretory cell; EP = excretory pore; GP = genital primordium; V = vulva.

TABLE 2.—*de Man's proportional measurements of* C. sweeti

No.	Stage of development of larvae	α Total length / Body width	β Total length / Esophagus length	γ Total length / Tail length	Vulva or genital primordium per cent of body length from anterior end
1	1st stage larva	17	3.6	6	58 (GP)
2	Late 1st stage larva, molting	19	3.6	7	59 (GP)
3	Early 2nd stage larva	18	3.6	12	57 (GP)
4	2nd stage larva, molting..	14	2.6	14	55 (GP)
6	3rd stage larva (from experimentally infected cyclops)	16	2.2	14	65 (GP)
7	Developed 3rd stage larva in 2nd intermediate host (natural infection)	22	2.3	18	63 (GP)
9	Late 4th stage larva, molting (natural infection from intestine final host)	27	3.1	22	56 (V)
10	Early 5th stage larva (natural infection from intestine final host)	33	3.8	33	59 (V)

Same number of specimens measured as in table 1.
GP = genital primordium ; V = vulva.

Description. Body 0.19 to 0.28 mm long and 0.012 to 0.018 mm wide. $\alpha = 15$ to 24, $\beta = 3$ to 5, $\gamma = 5$ to 7, genital primordium 53 to 65 per cent. Transverse cuticular striations fine; stoma small and narrow; characteristic dorsal triangular denticle present on the anterior extremity (fig. 1) ; amphids pore-like; esophagus, consisting of two parts, anterior muscular portion 0.027 to 0.062 mm in length and posterior glandular portion 0.012 to 0.030 mm in length (fig. 1) ; nerve ring 0.025 to 0.045 mm from cephalic extremity; excretory cell 0.080 to 0.121 mm from cephalic extremity, at about level of proximal one-fourth of intestine; excretory pore not distinct, apparently situated slightly anterior to the excretory cell nucleus (fig. 1) ; intestine consisting of 16 to 24 cells arranged in transverse rows of two to three cells (fig. 1) ; genital primordium 0.11 to 0.17 mm from the anterior extremity, consisting of four cells arranged in a longitudinal row and situated close to the intestinal wall; anus 0.15 to 0.22 mm from anterior extremity; phasmids large, pouch-like, slightly posterior to anus (fig. 1) ; tail 0.033 to 0.046 mm long terminating with a bluntly pointed tip.

In only a few of the first stage larvae of *C. sweeti* studied was it possible to differentiate the anterior muscular from the posterior glandular portion of the esophagus; in the majority of the other specimens the general appearance is indicated in fig. 2. Since this demarcation between the muscular and glandular esophagus has been observed in only a few preserved specimens of larvae, this observation needs confirmation.

Late first stage larva undergoing the first molt (Plate I, fig. 4). The first molt appears to take place 24 to 36 hours after the larva gains entrance into the body cavity of the cyclops. It is extremely difficult to recognize this molt since it occurs at such a short interval after infection; the exuviae are very thin and lie quite close to the cuticle of the larva. Of several specimens examined this molt was distinct in only one.

Description. The different measurements of the larva at this stage are given in tables 1 and 2. The general structure of the larva is about the same as the early first stage larva, with the following differences:

1. The anterior end is globular and devoid of the dorsal triangular denticle which appears to be lost with the exuviae (fig. 4).
2. The stoma is wider and more distinct.
3. The demarcation between the muscular and glandular portion of the esophagus is slightly more distinct.
4. The total number of cells in the intestine is about 35 cells.
5. The posterior end is thicker and more bluntly pointed.

Early second stage larva (Plate I, figs. 3 and 5). Only one specimen was available for study. This was obtained from the body cavity of cyclops, about 52 hours after infection.

Description. The different measurements of this specimen are given in tables 1 and 2. The following differences were noticed between this stage and the previous late first stage:

1. The stoma is very distinct and rectangular in shape (fig. 3).
2. A pair of very prominent esophageal nuclei is present (fig. 3).
3. The excretory pore is distinct and situated slightly posterior to the nerve ring (fig. 3).
4. The tail is short compared to the total body length, and is bluntly pointed at the tip (fig. 5).

Late second stage larva undergoing the second molt (Plate II, figs. 6 and 7). The larva undergoes the second molt between the 5th and 7th day during hot weather (90° to 102° F.) and between the 8th and 12th day during the cooler weather (55° to 70° F.). This molt unlike the first, was easily recognized and observed in a number of specimens.

Description. Average measurements of three specimens are given in tables 1 and 2. This stage resembles the third stage larva very closely but differs from the first stage larva in the following respects:

1. The stoma is completely formed; buccal cavity partially chitinized and separates the imperfectly chitinized jaws from the esophagus (fig. 7). The molted portion of the old stoma and esophagus is present in the anterior portion of the exuviae to be cast off.
2. The amphids are situated at the anterior margin between the two pairs of partially chitinized quadrilateral plates.
3. The external circle of two pairs of papillae is conspicuous (fig. 7).
4. The demarcation between the muscular and glandular esophagus is very distinct (fig. 7).
5. The excretory pore is distinct and situated slightly posterior to the nerve ring.
6. The intestine consists of 40 to 50 cells arranged in transverse rows of 2 to 3 cells each.
7. The genital primordium consists of four cells as in the previous stages. It is elliptical, nearly as long, but twice as broad, as in the earlier stages.
8. The rectum is just undergoing the molt (fig. 6).
9. The tail is short compared to body length and covered by the posterior portion of the exuviae to be cast off. Three characteristic mucrones are present at the tip (fig. 6).

Third stage larva (from experimentally infected cyclops) (Plate II, figs. 8, 9, and 10). Specimens of cyclops were infected with the first stage larvae of *C. sweeti* and had remained alive in the laboratory for over three weeks. The description is of larvae obtained from them.

Description. Average measurements of 10 specimens are given in tables 1 and 2. Body 0.47 to 0.65 mm long and 0.032 to 0.040 mm wide; $\alpha = 14$ to 18; $\beta = 2.0$ to 2.4; $\gamma = 12$ to 16; genital primordium 61 to 70 per cent; stoma, 0.030 to 0.037 mm long, well formed; buccal cavity partially chitinized, separating imperfectly chitinized jaws from esophagus; 8 to 11 partially chitinized, beaded ridges of almost the same length, extend from anterior margin to about the level of buccal cavity; amphids situated at anterior margin, between two pairs of partially chitinized quadrilateral plates; two pairs of papillae of external circle conspicuous (fig. 10); esophagus with anterior muscular portion 0.14 to 0.17 mm in length and posterior glandular portion 0.09 to 0.12 mm in length, demarcation between the two portions quite distinct; nerve ring 0.06 to 0.09 mm from anterior extremity; excretory pore slightly posterior to nerve ring; excretory cell conspicuous, situated at about level of base of muscular portion of esophagus (fig. 8); intestine consisting of 48 to 56 cells, containing large number of yellowish brown granules and transparent globules; genital primordium ellipsoidal in shape, 0.30 to 0.41 mm from anterior extremity, containing about eight cells nearly as long but about twice as broad as those of first stage larva (fig. 8); anus 0.43 to 0.61 mm from anterior extremity; tail 0.035 to 0.051 mm long, with three characteristic unequal mucrones present at tip, the mediodorsal being noticeably stouter than the other two (fig. 9); phasmids circular, posterior to anus (fig. 9).

Third stage larva found in intestines of B. puckelli *and* L. thermalis *under natural conditions* (Plate III, fig. 11). In the intestines of *B. puckelli* and *L. thermalis,* third stage larvae in the same stage of development as those found in experimentally infected cyclops, and also a few that appeared to have undergone further development in the fish, were found under natural conditions. Measurements and description given are of the latter forms that appeared to have undergone further developmental changes in the fish host.

Description. In structure this larva resembles very closely the third stage larva from experimentally infected cyclops except for the following differences:

1. It is about one and a half times as long and nearly as broad as the third stage larva from cyclops.
2. Jaws, buccal cavity, quadrilateral plates, and longitudinal ridges are nearly completely chitinized while in third stage larva from cyclops they are partially chitinized (fig. 11).
3. Ratio between length of anterior portion and posterior portion of esophagus is less than that in third stage larva from cyclops, and approximates more that of later developmental stages.
4. Genital primordium consists of eight cells, but is nearly one and a half times size in third stage larva from cyclops.
5. Intestine contains larger numbers of cells (about 80) than in third stage larva from cyclops.

Third stage larva undergoing third molt. In spite of repeated careful search for the third stage larva of *C. sweeti* undergoing the third molt, none has been secured from either intermediate or definitive hosts. Hence neither larval structure at this stage of development nor host in which the third molt takes place, could be determined. It seems likely that the third molt takes place in the intestine of the second intermediate host.

Fourth stage larva undergoing fourth molt from intestines of O.

gachua *infected under natural conditions* (Plate III, figs. 15 and 16). Out of several specimens of *O. gachua* that were examined only a single specimen of *Camallanus* larva undergoing (?) the fourth molt was obtained. Judging from the structure of the old stoma which was just being cast off and of the new one just being formed, it was thought at first to be undergoing the third molt. However, structure of genital primordium and already formed vulva suggested further development than the third stage and it is described as undergoing the fourth molt.

Description. Measurements are given in tables 1 and 2. The following distinctive developmental changes were observed:

1. Both the old stoma (resembling that of early third stage larva) just being shed, and the new one resembling that of the adult just being formed (fig. 15).
2. Number of cells in intestines has increased to about 120.
3. Genital primordium is conspicuous and considerably increased in size. Its length is 10 to 12 times and breadth 3 to 4 times that of the late third stage larva.
4. Vulva, vagina, and uterus are just being differentiated and sex can be easily recognized.
5. Tail is short and three characteristic mucrones are present at tip (fig. 16).

Early fifth stage larva (Plate III, fig. 17). Only one specimen was found in the intestines of *O. gachua* infected under natural conditions.

Description. The different measurements of the specimen are given in table 1. In general structure this stage resembles that of adult *C. sweeti* (Moorthy, 1937), except for the following differences:

1. Stoma is only partially chitinized and new longitudinal ridges are just being formed (fig. 17).
2. Intestine consists of about 200 cells.
3. Vulva and vagina are fully formed, but lips of vulva are not so prominent and do not project out from body as in adult.
4. Anterior and posterior portion of uterus is just being differentiated, the anterior end terminating in a long tubular ovary and the posterior in a small rudimentary ovary.

DISCUSSION OF LIFE HISTORY

In some of the specimens of *O. gachua,* a few of the dead adult females of *C. sweeti* that had completely emptied their uteri of larvae were present; and in a few others, living first stage larvae were found in the lower half of the intestine. It is suggested from these observations that under natural conditions the adult female worms discharge the larvae into the lumen of the intestine of *O. gachua,* the worms dying after all the larvae have been discharged. On reaching the water in the feces of the fish, the larvae are swallowed by a suitable species of cyclops, possibly adult females of *M. leuckarti,* and reach the body cavity of cyclops about two to three hours after infection; about 24 to 36 hours later, these larvae undergo the first molt; and after 5 to 7 days during hot weather or 8 to 12 days in the cooler weather they undergo the second molt. If at this

stage the infected cyclops are ingested by a suitable freshwater fish, *B. puckelli* or *L. thermalis,* they are killed by the digestive secretions, e.g., the bile, and the larvae are activated; on being released from the cyclops certain further development including possibly the third molt of the larvae takes place in the intestine of the fish. As has been stated, specimens of larvae undergoing the third molt have not been found, either in the second or in the final host. The infection of the final host possibly takes place by its feeding on the second intermediate host. The larvae undergo the fourth molt in the intestines of the final host where a distinct change takes place in the stoma from the early larval *Paracamallanus* type to the adult *Camallanus* type. After this molt the larvae become sexually mature. Copulation appears to take place in the first half of the intestine, as large numbers of males and females were usually found in that region. The impregnated females migrate to the lower half of the intestines where they discharge the larvae, which on reaching the water in the feces of the fish, are ingested by cyclops and the life cycle is started again.

In the laboratory it has so far been possible to follow the life history of *C. sweeti* only in the first intermediate host, the cyclops. Since both the second intermediate and final host are very heavily infected with this parasite under natural conditions in the locality where the investigation was conducted, it was not possible to follow the life history of the parasite in these hosts in the laboratory. Structural identity of larval forms obtained from the second intermediate and final host, with those obtained from cyclops experimentally infected with first stage larva of *C. sweeti,* give adequate support to the life history suggested.

Since it was not possible to follow the life history of *C. sweeti* in the second intermediate and final hosts in the laboratory, it is difficult at the present stage of the investigation to estimate how far the intervention of the second intermediate host is necessary for the infection of the final host and whether or not the final host can become infected by feeding directly on the first intermediate host. The presence of the third stage larva in the intestines of the definitive host (3 per cent) showing the same stage of development as in cyclops suggests that the final host gets the infection by feeding directly on cyclops; but the presence in the intestines of the final host also of the later developed stage found in the second intermediate host suggests that the early third stage larva is possibly introduced by the final host feeding on the second intermediate host which harbors both the stages of the larvae and not by feeding directly on cyclops. In addition, it has been observed in the field that *O. gachua* does not feed to any great extent on cyclops directly, but feeds voraciously on the smaller cyclops-eating fish, especially *L. thermalis,* in which the highest percentage (42 per cent) of the late third stage larval infection of *C. sweeti* was observed under natural conditions. The largest percentage

(10 per cent) of the early developmental stage of *C. sweeti* found in the intestine of *O. gachua* are structurally identical with the stage of maximum development of *C. sweeti* in the intestines of the second intermediate host. Adult *C. sweeti* have so far been found only in *O. gachua* which feeds essentially on the second intermediate host, and not in any of the other species of fish that feed voraciously on cyclops and other copepods. It is suggested from these observations that under natural conditions the infection of the final host does not take place without the intervention of the second intermediate host.

The most interesting feature in the life history of *C. sweeti* is the transformation of the stoma from the *Paracamallanus* type found in the third stage larva to the *Camallanus* type found in the adult when the larva undergoes the fourth molt in the intestines of *O. gachua.* As has already been pointed out (Moorthy, 1937b), the presence or absence of a large buccal cavity does not appear to be a satisfactory generic character and it is possibly superfluous to keep *Paracamallanus* as a separate genus.

UNDETERMINED FORMS OF CAMALLANID LARVAE

Camallanid larva from naturally infected cyclops (Plate IV, figs. 18, 19, and 20). As has been stated, the third stage *Camallanus* larva obtained from a single specimen of cyclops infected under natural conditions seems to be structurally different from the third stage larva of *C. sweeti* obtained either from the second intermediate host or from cyclops experimentally infected in the laboratory. Since this was the only camallanid larva so far found in cyclops collected from ponds containing large numbers of *O. gachua* infected heavily with *C. sweeti,* a brief description of this larva is given. The adult of this species of *Camallanus* has so far not been found in any of the fish hosts examined. It is possible that some other aquatic animal (turtle ?) is the final host of this species of *Camallanus.*

Description. Body 1.09 mm long by 0.07 mm wide; $\alpha = 16$, $\beta = 2.8$, $\gamma = 23$, genital primordium 66 per cent; stoma 0.046 mm long, consisting of an almost completely chitinized buccal cavity and well developed paired jaws (fig. 19); 14 longitudinal ridges, not beaded and of unequal length, extend from the cephalic extremity to the upper margin of the chitinous ring; two pairs of papillae of external circle conspicuous; amphids situated at the anterior margin between the two pairs of completely chitinized quadrilateral plate; cuticular striations transverse deep, distinct, and about 4 μ apart; esophagus with anterior muscular portion 0.26 mm long and posterior glandular portion, 0.14 mm long, the demarcation between the two portions quite distinct (fig. 18); nerve ring 0.13 mm from the anterior extremity; excretory pore slightly posterior to nerve ring; excretory cell nucleus at base of muscular portion of esophagus; intestine consisting of 65 cells arranged in transverse rows of three to four cells each, containing yellowish brown granules and transparent globules (fig. 18); genital primordium, 0.72 mm from the anterior extremity; consists of four cells arranged longitudinally (fig. 18); anus 1.05 mm from the anterior extremity; phasmids circular, situated slightly posterior to anus (fig. 20); tail 0.46 mm long; three very prominent equal mucrones are present at the tip (fig. 20).

This larva differs from the third stage larva of *C. sweeti* obtained from experimentally infected cyclops and also the early third stage larva obtained from the intestines of the second intermediate host infected under natural conditions as follows:

1. The chitinization of the buccal cavity, the jaws, and the longitudinal ridges is nearly complete, while it is only partial in larvae obtained from cyclops or fish.
2. The buccal cavity is only half as long as in the other forms.
3. The larvae are nearly twice as long and broad as those obtained from experimentally infected cyclops.
4. The transverse cuticular striations are deeper, more pronounced, and farther apart.
5. The genital primordium is longer and narrower, situated more posteriorly, and has only four cells.
6. The three mucrones at the tip of the tail are almost equal in size, while they are unequal in *C. sweeti,* the mediodorsal one being noticeably stouter and longer than the other two.
7. The ridges at the anterior margin of the stoma are 14 in number, unequal and are not beaded, while there are usually not more than 11 ridges of about the same length, every one of them distinctly beaded, in the third stage larva of *C. sweeti.*

These differences in the structure of this larva suggest that it probably represents the third larval stage of a different species of *Camallanus.* The exact identification of the species was not possible as only one larva was available for study and its structure did not agree with any of the known adults of this genus.

Encysted camallanid larva found in the second intermediate host (Plate III, figs. 12, 13, and 14). In about 5 per cent of *B. puckelli* and 18 per cent of *L. thermalis,* infected under natural conditions, an encysted camallanid larva (fig. 12) was found in the body cavity of the fish, in addition to the third stage larvae of *C. sweeti* that were found in the intestinal lumen. These encysted forms have also been found in about 2 per cent of *O. gachua.* In the smaller species of fish these forms were found encysted in the body cavity of the fish lying loosely attached to the intestines; in *O. gachua* they were always found in the lumen of the foregut, some being enveloped in the cyst and others being set free from it. This observation suggests that the encysted form is one of the early developmental stages of a camallanid, which when introduced into the intestines of *O. gachua* excysts and possibly does not undergo any further development. Structurally these forms appear to be different from any of the developmental stages of *C. sweeti* so far known.

Description. Body 1.1 to 1.6 mm in length, 0.05 to 0.06 mm in width; $\alpha = 17$ to 26, $\beta = 2.8$ to 3.9, $\gamma = 16$ to 23, genital primordium 50 to 64 per cent; stoma 0.035 to

0.048 mm in length; buccal cavity very narrow, being only half as long as that found in the third stage larva; longitudinal ridges observed in the later developmental stages of *C. sweeti* not present; two pairs of papillae of external circle, conspicuous; amphids situated at the cephalic extremities between the two pairs of chitinized quadrilateral plates (fig. 14); esophagus with anterior muscular portion 0.19 to 0.22 mm long and posterior glandular portion 0.16 to 0.22 mm long; nerve ring 0.09 to 0.11 mm from the cephalic extremity; excretory pore slightly posterior to nerve ring; intestine with 100 to 120 cells arranged in transverse rows of three to four cells each, containing a large number of yellowish brown granules; genital primordium nearly three to four times as long and about twice as broad as that of the third stage larva; anus 1.0 to 1.5 mm from the anterior extremity; tail 0.063 to 0.081 mm long, bluntly conoid, without mucrones (fig. 13).

The peculiar shape of the stoma, neither resembling the early larval stages nor that of the adult of *C. sweeti,* the entire absence of longitudinal ridges in the stoma and the absence of the characteristic mucrones at the tip of the tail which are present both in the early stages and in the mature adults of *C. sweeti,* suggest that these encysted forms represent a developmental stage of a different species of camallanid. Since *C. sweeti* is the only species of adult camallanid that has so far been found in *O. gachua,* it appears to be probable that the adult of this species is present in some other final host. Beyond excystation no further development of this encysted form is observed in *O. gachua.* The presence of these forms in *O. gachua* is possibly only accidental due to its feeding on *B. puckelli* and *L. thermalis* that harbor this encysted form.

SUMMARY

A description is given of the different developmental stages of *C. sweeti* (except that of the third stage larva undergoing the third molt) as they occur in the intermediate and the final host. There is also described a camallanid larva of unknown species found in the body cavity of naturally infected cyclops, and an encysted camallanid larva of unknown species found in the peritoneal cavity of *B. puckelli* and *L. thermalis.* It is suggested that in the life history of *C. sweeti* the intervention of two intermediate hosts, the cyclops and a freshwater fish (*B. puckelli* or *L. thermalis*) are necessary. Since under natural conditions there was heavy infection in the second intermediate and final host, it was not possible to follow the life history of *C. sweeti* in these hosts in the laboratory. It has been observed that fresh bile has identically the same effect on cyclops infected with *Camallanus* larva as on cyclops infected with *Dracunculus* larva, in both cases the cyclops being killed and the encysted larva activated.

Bibliography

Moorthy, V. N. 1935 The influence of fresh bile on guinea worm larvae encysted in cylops. Ind. Med. Gaz. **70**: 21.

————1937a A simple method of staining and mounting nematode larvae. J. Parasitol, **23**: 100–102.

————1937b *Camallanus sweeti,* a new species of Camallanidae (Nematode). J. Parasitol. **23**: 302–306.

Moorthy, V. N. and Sweet, W. C. 1936 Guinea-worm infection of cyclops in nature. Ind. Med. Gaz. **71**: 568.

EXPLANATION OF PLATES

Abbreviations

Amp. ....Amphid
C.P. ....Cephalic papillae
D. .....Dorsal denticle
Ec.n. ....Excretory cell nucleus
E.P. .....Excretory pore
G.P. .....Genital primordium
N.R. ....Nerve ring
N.S. ....New stoma just forming
O.N. ....Esophageal nuclei
O.S. .....Old stoma just being cast
Ph. .....Phasmid
St. .....Stoma

Plate I

Camallanus sweeti

Fig. 1. First stage larva: the general appearance observed in a few specimens in which the line of demarcation between the muscular and glandular esophagus was distinct.

Fig. 2. First stage larva, esophageal region: the general appearance observed in the majority of the larvae studied; here the line of demarcation between the muscular and glandular esophagus is not distinct.

Fig. 3. Early second stage larva: esophageal region.

Fig. 4. Late first stage larva undergoing the first molt: esophageal region.

Fig. 5. Early second stage larva: posterior region.

Plate II

Developmental stages of *Camallanus sweeti* from experimentally infected cyclops.

Fig. 6. Second stage larva undergoing the second molt: posterior region.

Fig. 7. Second stage larva undergoing the second molt: esophageal region, lateral view.

Fig. 8. Third stage larva: general lateral view.

Fig. 9. Third stage larva: tail, lateral view.

Fig. 10. Third stage larva: cephalic region, lateral view.

Plate III

Fig. 11. Late third stage larva obtained from the intestines of the second intermediate host: cephalic region.

Fig. 12. Encysted camallanid larva (sp. ?) found in the peritoneal cavity of the second intermediate hosts and also in the intestines of *O. gachua.*

Fig. 13. Tail of encysted camallanid larva (sp. ?).

FIG. 14. Cephalic portion of the encysted camallanid larva (sp. ?).

FIG. 15. Fourth stage larva of *C. sweeti* undergoing the fourth molt (from the intestines of *O. gachua*) ; shows the transformation of the *Paracamallanus* type of stoma to *Camallanus* type.

FIG. 16. Tail end of the fourth stage larva of *C. sweeti,* undergoing the fourth molt.

FIG. 17. Early fifth stage larva of *C. sweeti* (from the intestines of *O. gachua*) ; shows the *Camallanus* type of stoma and the longitudinal ridges at the anterior extremity, just forming.

PLATE IV

Third stage *Camallanus* larva (sp. ?) found in cyclops infected under natural conditions.

FIG. 18. General lateral view.

FIG. 19. Head, lateral view.

FIG. 20. Tail, lateral view.

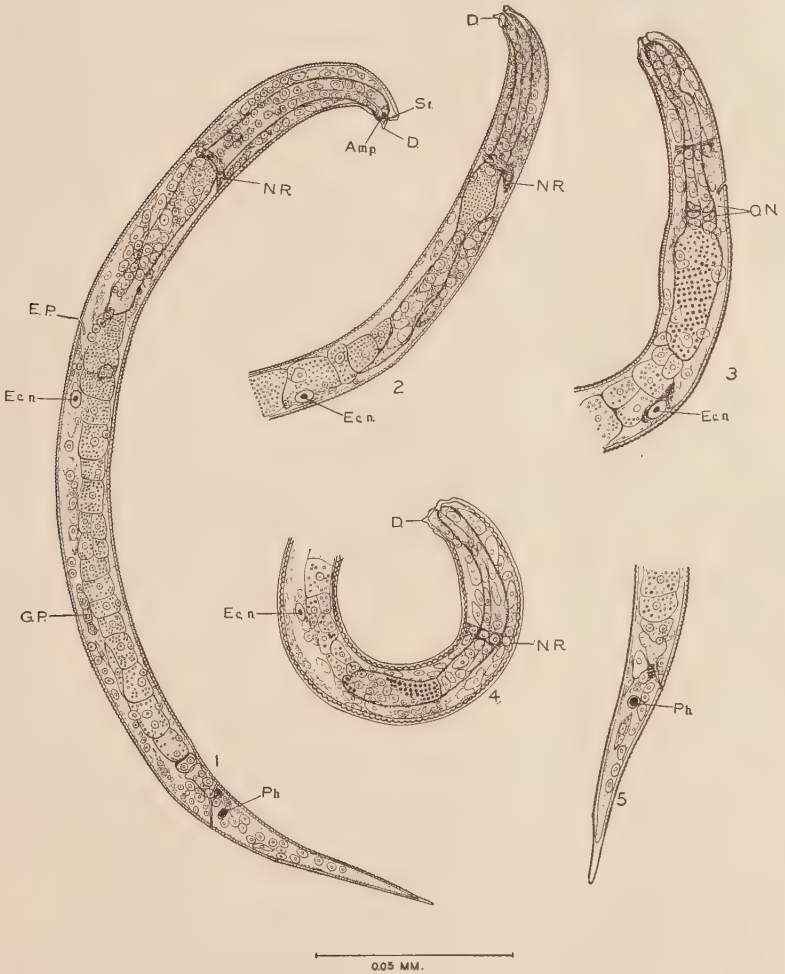


PLATE I

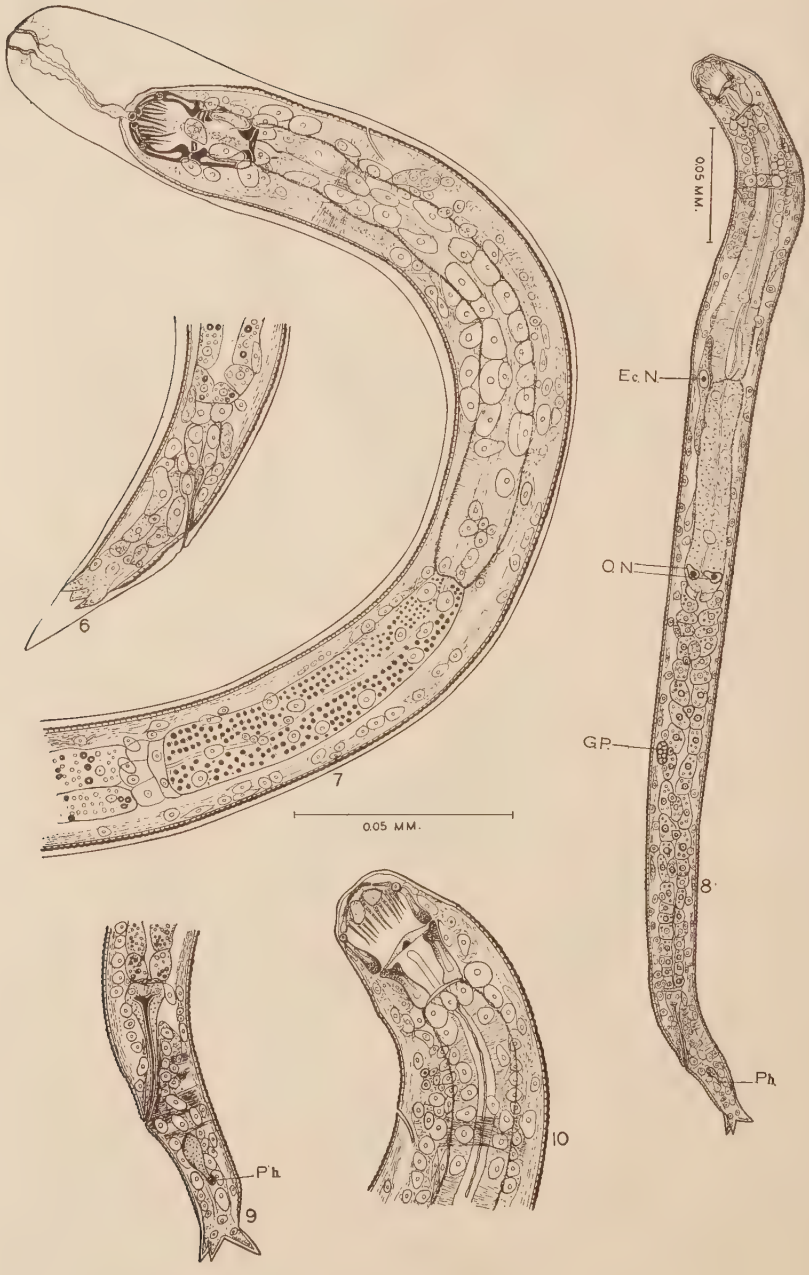
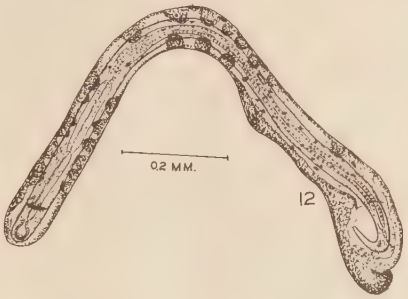
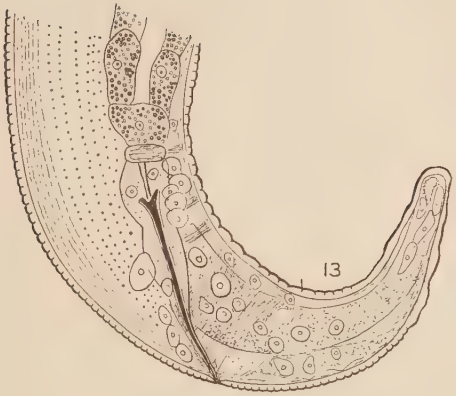
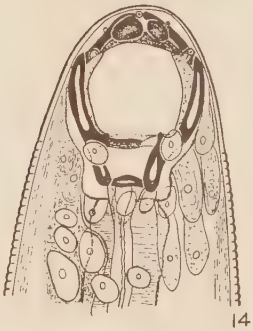
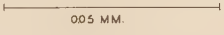


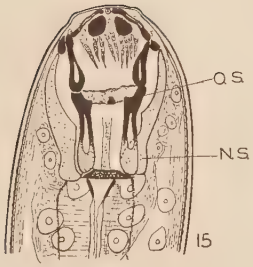
PLATE II











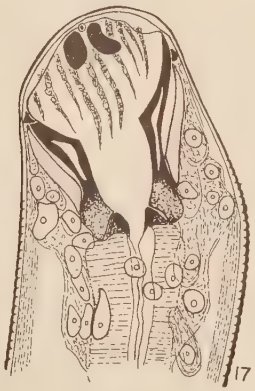


PLATE III

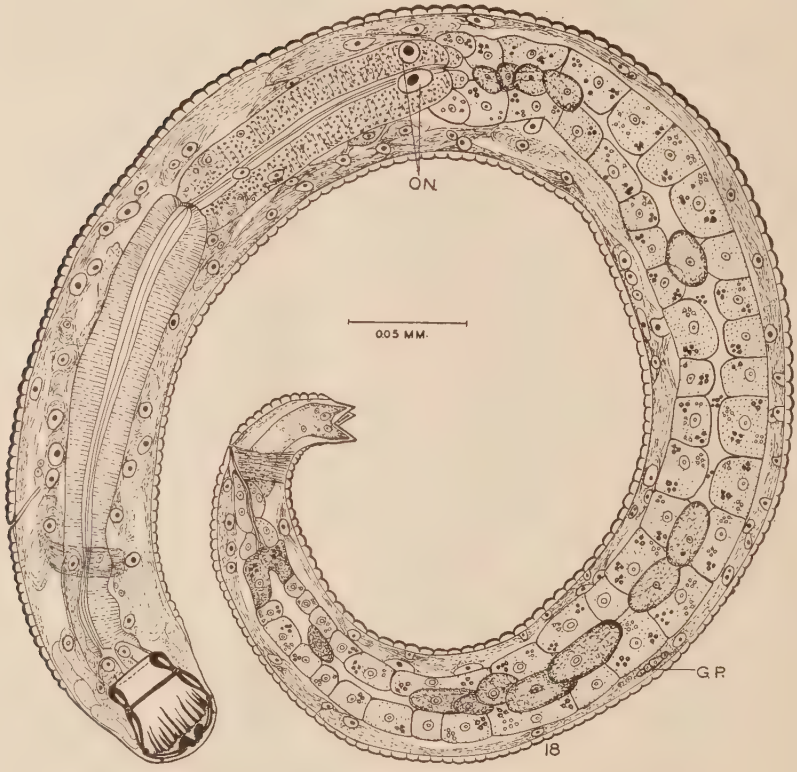


PLATE IV

A SYNOPSIS OF THE FLAGELLATE GENUS *COCHLOSOMA* KOTLÁN, WITH THE DESCRIPTION OF TWO NEW SPECIES[1]

BERNARD V. TRAVIS[2]

The generic name *Cochlosoma* was created by Kotlán (1923) to include a peculiar flagellate, *C. anatis,* which he found in the intestines of European domestic ducks. Kimura (1934) described a second species, *C. rostratum,* from domestic ducks in North America. His work contains an excellent and detailed description concerning the morphology of *C. rostratum.* The nomenclature of the various structures discussed in this paper is that used by Kimura. Tyzzer (1930) described two similar genera from the intestines of ruffed grouse. He erected a new family, COCHLOSOMIDAE, to include the type genus *Cochlosoma* Kotlán and his new genera *Cyanthosoma* and *Ptychostoma.*

Generic diagnosis: Body shape ovoidal, broadly rounded anteriorly and narrowly rounded posteriorly. Six flagella of unequal length, two of which are trailing and lie in a lateral groove, arise from a blepharoplastic complex at the anterior end of the body. Nucleus, spherical to slightly ellipsoidal, situated near the middle of the body. Two slender axial fibrils arise from the blepharoplastic complex, the more central and smaller one is the axostyle, the more lateral and larger one is the costa or chromatic basal rod. In an antero-ventral position is a large sucker organelle open on the left side and provided with a marginal fibril. The known species are parasites of birds.

Type species: Cochlosoma anatis Kotlán, 1923.

Systematic position. The genus *Cochlosoma* is a flagellate belonging to the order POLYMASTIGINA Blochmann. Systematically it seems to be a connecting link between the trichomonad flagellates of the MONOZOA and the *Giardia* of the DIPLOZOA. The sucker organelle, the presence of two axial fibrils, and the median position of the nucleus are giardia-like. The flagella, which originate from a blepharoplastic complex at the anterior end of the body, the axial fibrils which seem to be homologous with the costa and the axostyle, and the presence of one nucleus, are characters that suggest trichomonad affinities.

Economic status. Kotlán has briefly described the pathogenicity of *Cochlosoma.* In one case he noted that the intestinal wall of a bird was

Received for publication, December 20, 1937.

[1] Journal Paper No. J-519 of the Iowa Agric. Exper. Sta., Ames, Ia., Project 570. Iowa State College, Iowa Conservation Commission and American Wildlife Institute cooperating with the U. S. Bureau of Biological Survey.

[2] Formerly with the Iowa Agricultural Experiment Station; resigned September, 1935.

swollen and catarrhal at the point where a mass of the flagellates were attached, and that the intestinal contents were mixed with blood. Kimura was unable to attach any pathogenic significance to this organism. He noted inflamed intestinal tracts, but ascribed this condition to the bacteria rather than to the flagellates. The author has not observed any pathogenic disturbances either in the nine naturally infected wild ducks, the one domestic duck, in the Eastern robin, or in the American magpie studied by him. Controlled experiments are needed to demonstrate the effect of these flagellates on their hosts.

Cochlosoma anatis Kotlán, 1923.

(Figs. 2, 3; tables 1, 4)

TABLE 1.—*Correlation table of the length and width of 100 specimens of* C. anatis *Kotlán from wild mallards. Mean length, 8.4 μ; mean width, 4.9 μ.*

Width in microns	Length in microns											Total
	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5	
4.0	1	3		5	6	2						17
4.5		2	2	4	11							19
5.0	1	2	2	13	13	7						38
5.5				2	3	9	3	2		1		20
6.0						3			1	1	1	6
Total	2	7	4	24	33	21	3	2	1	2	1	100

This flagellate was described by Kotlán from the intestines of European domestic ducks. He also mentioned the occurrence of members of this genus in the birds *Nyroca ferruginea,* and *Fulica atra.* Kimura (1934) applied the name *C. rostratum* to the flagellates he found in the intestines of the white Pekin, a domesticated variety of the mallard, *Anas platyrhynchos* L., and the domesticated muscovy, *Cairina moschata* L. The author considers *C. rostratum* Kimura to be a synonym of *C. anatis* Kotlán. The reasons for this change will be presented later in this paper. Specimens have been studied from the cloaca, ceca, and large intestines of five wild mallard ducks, *Anas platyrhynchos platyrhynchos* L., one pintail duck, *Dafila acuta tzitzihoa* (Vieillot), two shoveler ducks, *Spatula clypeata* (L.) collected at Ruthven, Iowa, one lesser scaup, *Nyroca affinis* (Eyton), and one domestic mallard duck, *Anas platyrhynchos* L., examined at Ames, Iowa.

These flagellates are common residents of the cloaca, but they are frequently present in the large intestine and occasionally in the ceca. There is no evidence (Kimura, 1934) to indicate that the sucker disc is used as an organ of attachment such as is common in *Giardia.* The protruding tip of the axostyle appears to be the means of attachment to the intestinal cells (Kimura, 1934).

The body shape is broadly ovoidal in a dorsal or ventral view, broadly rounded at the anterior end, and tapering to a narrowly rounded posterior end. From a lateral view, the body is slender, with the dorsal surface

arched, the ventral side has a rather deep concavity or sucker organelle at the anterior end; posterior to the concavity is a subtriangular-shaped area or tail-piece. The sucker is clearly visible from a ventral view as a subround clear area at the anterior end of the body. The sucker is not circular in outline, as Kotlán has interpreted it, but instead, as Kimura has pointed out, it is open on the left side. The opening is closely associated with a rather deep longitudinal groove extending full length of the organism. A lateral tongue-like projection extends outward at the upper left margin of the sucker disc. A deeply staining fibril margins this sucker disc.

Kotlán described the axostyle as being composed of two "fibrilles." He thought that these arose from the basal granule or from a near-by granule. One of these fibrils may be considered to be homologous with the axostyle of *Monocercomonoides* and *Monocercomonas* (Travis, 1932). This fibril arises from a primary blepharoplast near the nucleus, curves to the right and passes to the posterior end of the animal where it projects 1 to 2 μ from the body. A rhizoplast connects the primary and the secondary blepharoplasts. The second fibril, the costa, arises from a quaternary blepharoplast, which is located to the left of the nucleus, and passes to near the posterior end of the animal. It does not project beyond the cytoplasm. A rhizoplast connects the tertiary and the quaternary blepharoplasts.

Kotlán described many "fibrilles," arising from the basal granule or "centroblepharoplast," which were directed backwards and six of which extended along the edge of the body. There are only six "fibrillen" or flagella in a typical trophozoite. The marginal flagellum on the sucker organelle arises from the quaternary blepharoplast and extends beyond the tongue-like projection on the open side near the disc. Two trailing flagella arise from the tertiary blepharoplast and lie in the longitudinal groove on the left side of the body. They are 7 to 20 μ in length. Three active flagella 10 to 15 μ in length, also arise from the tertiary blepharoplast.

Attached to the quaternary blepharoplast is a body that stains darkly with Heidenhain's iron-hematoxylin after fixation in Schaudinn's fluid. Kimura considered this structure to be homologous with the parabasal body of other flagellates. Ordinarily the parabasal apparatus in flagellates does not stain after fixation in Schaudinn's fluid, however, since the location and general appearance of this body is similar to the parabasal body of *Trichomonas,* the interpretation of Kimura is followed in this paper.

The nucleus, which is 1.2 to 3.0 μ in diameter, is located near the center of the body. In stained specimens, the internal morphology is seen with difficulty due to the presence of numerous cytoplasmic granules.

Living specimens have a peculiar swimming motion somewhat like that of *Giardia*. The trailing flagella beat rather slowly in the longitudinal groove, whereas the active flagella beat vigorously in unison on the left side. Since the flagella beat on the one side, the organism moves forward with an erratic, jerky motion. The body rotates on its long axis as it progresses with very little of the "dipping" motion of *Giardia*.

Kimura observed rod-shaped bacteria adhering to the body of the flagellates from certain of his domestic ducks. This was also true for the material from wild ducks studied by the author.

Reproduction in this species was recorded briefly by Kotlán. He observed longitudinal division and cysts with four or more nuclei, but he published no drawings either of divisional stages or of the cysts. No dimensions were given for the cysts. Cysts were not seen by the author.

Kimura reports longitudinal division as a mechanism of reproduction and figures one case. Divisional stages for this species were scarce on the author's slides. Occasional specimens showed two masses of chromatin material, a few had four rod-shaped masses which are considered to be chromosomes. The divisional stages were of the same type as those seen in the species from the magpie. A discussion of this method of reproduction will be given with the latter species.

Cochlosoma picae n. sp.

(Figs. 4, 5, 6, 7, 8, 9, 10; tables 2, 4)

Great numbers of this flagellate were present in the cloaca of one American magpie, *Pica pica hudsonia* (Sabine) (type host), collected at Bliss, Idaho, during the summer of 1932.

This species is similar to *C. anatis,* but differs principally in the smaller size and in the absence of cytoplasmic granules that stain with haematoxylin. The six flagella vary from 7 to 14 μ in length. The posterior end is more narrowly rounded than is *C. anatis*.

TABLE 2.—*Correlation table of the length and width of 100 specimens of* C. picae *n. sp. from the American magpie. Mean length, 6.1* μ*; mean width, 3.8* μ.

Width in microns	Length in microns						Total
	5.0	5.5	6.0	6.5	7.0	7.5	
3.0		2					2
3.5	1	10	20	4	7	1	43
4.0	4	7	24	7	7		49
4.5			1	2	3		6
Total	5	19	45	13	17	1	100

Occasionally a few bacteria were observed clinging to the bodies of this species, which appeared to be identical to those seen on *C. anatis*.

The posterior end of *C. picae* can be flexed in the manner similar to that in *Giardia*. Frequently the posterior end of fixed specimens may be

seen bent backwards as in fig. 10. In living specimens the tail-piece is sometimes seen to whip rapidly up and down.

No vacuoles were observed in stained specimens. No cysts were observed. The cytoplasmic granules characteristic of *C. anatis* are absent in this species.

The thin walled nucleus is round, 0.5 to 1.0 μ in diameter. The chromatin material in the resting forms is usually seen as deeply staining scattered granules.

Dividing forms were present in the smears from the magpie. Only longitudinal division, such as that possibly seen by Kotlán in *C. anatis* and that described by Kimura, was observed. The nuclear wall stains feebly and appears to remain intact during the reproductive process. The chromatin forms into a rod-shaped mass and then divides into four rod-shaped granules or chromosomes arranged in an equatorial plate in the center of the nucleus. The nucleus elongates and two of the chromosomes move to each end of the elongated nucleus (fig. 8). A centrodesmose is frequently seen connecting the chromatic granules (fig. 6). The nuclear wall constricts in the middle and forms two daughter nuclei (figs. 5, 7). The chromatic granules round up and then break into many small granules that fill the nucleus. The daughter blepharoplasts separate and migrate apart a short distance. The body begins to split at the anterior end; the split extends backwards until two daughter organisms result (figs. 5, 7).

In table 4 are included the measurements of *C. picae, C. turdi,* the original measurements of *C. anatis* Kotlán, measurements of this latter species by the author, and measurements of *C. rostratum* Kimura.

Type slides of new species are in the author's collection, in the Department of Zoology, Iowa State College, and in the U. S. National Museum.

Type slide for *C. picae,* U. S. Nat. Mus. No. 22612.

Cochlosoma turdi n. sp.

(Fig. 11; tables 3, 4)

This small flagellate was found to be rather numerous in the cloaca of one of three Eastern robins, *Turdus migratorius migratorius* L. (type host), collected at Ames, Iowa, during the spring of 1932.

Like *C. anatis,* this species has a large sucker disc and cytoplasmic granules. The small size, however, will separate it from the duck flagellate. The dimensions of *C. turdi* are like those of *C. picae,* but the robin flagellate can be distinguished from the latter by the presence of cytoplasmic granules and a larger sucker disc.

The six flagella vary in length from 4.5 to 9.0 μ. The nucleus appears

to have a thicker wall than does *C. anatis* or *C. picae.* The sucker disc which is more inconspicuous than in the two latter species is only seen clearly in a side view.

Of the three species, this is the most difficult for cytological observation. There is little chromatin material in any of the species to take the stain. In the material at hand, the robin flagellate stains most feebly. Further observations on new material may reveal additional morphological characters not seen by the writer.

Type slide for *C. turdi* U. S. Nat. Mus. No. 22613.

TABLE 3.—*Correlation table of the length and width of 100 specimens of* C. turdi *n. sp. from the Eastern robin. Mean length, 6.0* μ*; mean width, 3.8* μ.

Width in microns	Length in microns								Total
	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	
2.5 ...		1							1
3.0 ...	1	4	5	6	2				18
3.5 ...	1	2	6	15	7	1			32
4.0 ...		2	9	12	5	4	1	1	34
4.5 ...			1	3	5				9
5.0 ...			1		2	3			6
Total ...	2	9	22	36	21	8	1	1	100

DISCUSSION OF THE STATUS OF *C. anatis* KOTLÁN AND *C. rostratum* KIMURA

The work of Kotlán indicates that he did not make a detailed study of the morphology of the duck flagellate, *C. anatis* (see fig. 2). Conversely Kimura had carefully examined (see fig. 1) the flagellate which he calls *C. rostratum.* Based on their work and that of the author, it seems advisable to consider *C. rostratum* a synonym of *C. anatis,* for the following reasons:

1. Both the European and North American domestic mallards and their varieties have been derived primarily from the domestication of wild mallards, *Anas platyrhynchos platyrhynchos* L. Parasitologists therefore might expect the domesticated birds to harbor intestinal flagellates indistinguishable from those found in the wild mallards.

2. The ranges in size reported by Kotlán for *C. anatis* of 5 to 12×3 to 6 μ and by Kimura of 6.1 to 10.0×3.9 to 6.7 μ might well be considered only a variation of size not only of a single species, but the small differences could easily be the result of different individuals making the measurements, or of a variation in size due to host differences.

3. The differences in the morphology of the flagellates reported by the two authors appears, after a careful study of the specimens from both wild and domesticated mallards, to be merely differences in the interpretation of the structures in the respective parasites.

TABLE 4.—*A table showing the dimensions in microns of* C. picae, *from the American magpie,* C. turdi, *from the Eastern robin,* C. anatis *from North American ducks,* C. anatis *from European ducks, and* C. rostratum *from North American ducks.*

	C. picae from magpie	*C. turdi* from robin	*C. anatis* from N. Amer. ducks	*C. anatis* from European ducks (after Kotlán)	*C. rostratum* from N.A. ducks (after Kimura)
Length					
Mean	6.1	6.0	8.4	....	8.1
Range	5.5–7.5	4.5–8.0	6.5–11.5	5–12	6.1–10.0
Width					
Mean	3.8	3.8	4.9	....	5.5
Range	3.0–4.5	2.5–5.0	4.0–6.0	3– 6	3.9–6.7
Nucleus					
Mean	0.7	1.4	2.1	....	.....
Range	0.5–1.0	1.3–1.7	1.2–3.0	....	1.2–1.7
Length of sucker					
Mean	2.7	3.3	4.8	Diameter 1/3 to 1/2 body length	4.2
Range	2.0–3.0	2.5–4.6	4.5–5.0		3.3–4.4
Width of sucker					
Mean	1.9	3.0	3.9	....	3.9
Range	1.5–2.0	2.9–4.1	3.4–4.1	....	3.3–5.0

SUMMARY

Cochlosoma rostratum Kimura is believed to be a synonym of *C. anatis* Kotlán for the following reasons: (1) European and North American domesticated ducks have been derived of the same species of wild duck, (2) the small difference in size reported by the two authors is not sufficient evidence to separate the two flagellates into two species, and (3) the differences in morphology are considered to be differences in interpretation of structures. *C. anatis* was studied from four species of wild ducks, mallard, *Anas platyrhynchos platyrhynchos* (L.), shoveler, *Spatula clypeata* L., pintail, *Dafila acuta tzitzihoa* (Vieillot), lesser scaup, *Nyroca affinis* (Eyton), and from the domesticated mallard, *Anas platyrhynchos* L.

A new species, *Cochlosoma picae,* is described from the American magpie, *Pica pica hudsonia* (Sabine). It differs from *C. anatis* chiefly in that it is much smaller, and is without the cytoplasmic granules.

A second new species, *C. turdi,* is described from the Eastern robin, *Turdus migratorius migratorius* L. It differs from *C. anatis* in that it is smaller, and from *C. picae* in the presence of cytoplasmic granules and the larger sucker disc.

BIBLIOGRAPHY

KIMURA, G. G. 1934 *Cochlosoma rostratum* sp. nov., an Intestinal flagellate of domesticated ducks. Tr. Am. Micr. Soc. **53**: 102–116.

KOTLÁN, A. 1923 Zur Kenntnis der Darmflagellaten aus der Hausente und andern Wasservogeln. Centr. Bakt. I Abt. Orig. **90**: 24–28.

TRAVIS, B. V. 1932 A discussion of synonymy in the nomenclature of certain insect flagellates, with the description of a new flagellate from the larvae of *Ligyrodes relictus* Say (Coleoptera-Scarabaeidae). Iowa State Coll. J. Sc. **6**: 317–323.

TYZZER, E. E. 1930 Flagellates from the Ruffed Grouse. Am. J. Hyg. **11**: 56–73.

Explanation of Plate

magnification × 5000

Cochlosoma

1. Diagrammatic drawing naming the structures (after Kimura).

Cochlosoma anatis Kotlán, 1923

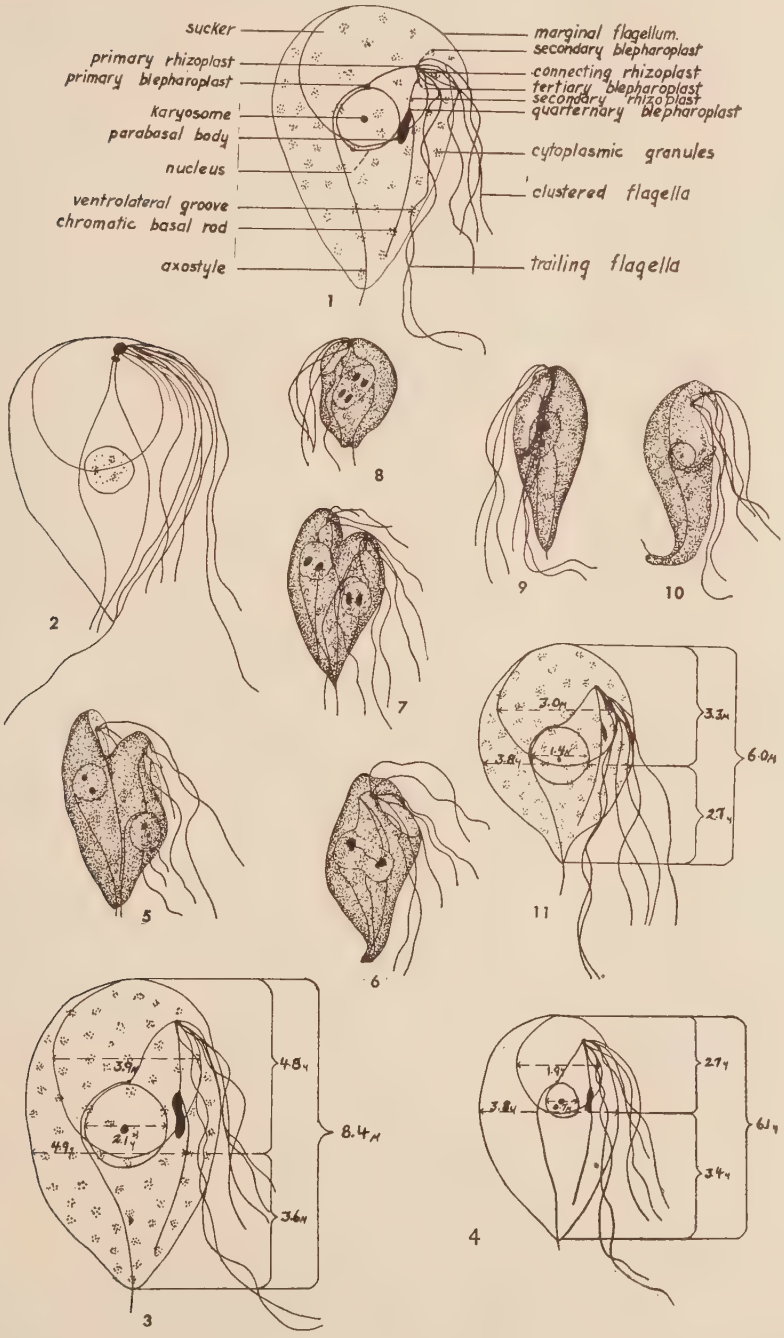
2. Trophozoite (after Kotlán).
3. Ideal drawing based on measurements of 100 specimens.

Cochlosoma picae n. sp.

4. Ideal drawing based on the measurements of 100 specimens.
5. Telophase with two chromosomes in each daughter nucleus.
6. Anaphase with two pairs of chromosomes and a centradesmose.
7. Telophase with two rod-shaped chromosomes in each daughter nucleus.
8. Late metaphase with four chromosomes.
9. Mesophase with two chromosomes.
10. Lateral view showing tail-piece flexed.

Cochlosoma turdi n. sp.

11. Ideal drawing based on the measurements of 100 specimens.


sucker
primary rhizoplast
primary blepharoplast
karyosome
parabasal body
nucleus
ventrolateral groove
chromatic basal rod
axostyle
marginal flagellum
secondary blepharoplast
connecting rhizoplast
tertiary blepharoplast
secondary rhizoplast
quarternary blepharoplast
cytoplasmic granules
clustered flagella
trailing flagella
1
2
3
4
5
6
7
8
9
10
11
3.0μ
3.3μ
6.0μ
1.4μ
3.8μ
2.7μ
3.9μ
4.8μ
8.4μ
2.1μ
4.9μ
3.6μ
1.9μ
2.7μ
3.8μ
6.1μ
3.4μ

DESCRIPTION AND LIFE HISTORY OF THE NEMATODE *DRACUNCULUS OPHIDENSIS* N. SP., WITH A REDESCRIPTION OF THE GENUS*

Sterling Brackett

During the summer of 1934, nematodes of the genus *Dracunculus* were found in a number of garter snakes (*Thamnophis sirtalis*) from the vicinity of the Biological Station of the University of Michigan, located on Douglas Lake in the northern tip of the southern peninsula of Michigan. These worms were found again during the summers of 1935, 1936, and 1937. Since morphological studies showed that these worms represent a species as yet undescribed, the name *Dracunculus ophidensis* is proposed for them.

Dracunculus ophidensis n. sp.

(Figs. 3, 4, 6 and 8)

Specific diagnosis: Genus *Dracunculus*. Oral opening surrounded by an internal circle of six papillae, of which the interno-laterals are most prominent, and an external circle of four double papillae. Amphids prominent and posterior to interno-laterals. Cuticular ridge on head prominent. Anterior muscular end of esophagus short. Glandular section of esophagus long and divided into a short anterior section separated from a much longer posterior section by a constriction. Nerve ring surrounds digestive tube at this constriction. Deirids just posterior to nerve ring.

Male: Up to 16 mm long by 0.17–0.20 mm in diameter. Nine pairs of genital papillae, 5 preanal and 4 postanal; pairs (numbering from posterior forward) 1 and 2 subventral (occasionally those of one side may be closer together than those of the other), pair 3 ventrolateral, pair 4 ventral, pairs 5–9 in a series just anterior to the anus (fig. 3). Measurements of a specimen 13.8 mm in length: anterior end to anterior tip of testis 2.7 mm; esophagus 7.7 mm; spicules slightly subequal, protruding about half their length, right 0.554 mm, left 0.523 mm; gubernaculum about 0.90 mm long; tail 0.17 mm.

Female: Up to 250 mm long. Arrangement of the cephalic papillae similar to that of male. Cuticular ridge absent in young developing forms but present in older specimens. Many of the structures atrophied or reduced in gravid individuals. Vulva at or posterior to middle of body. Measurements of a specimen 24.6 mm long: esophagus 9.9 mm; anterior ovary 5.1 mm; vagina 1.4 mm; vulva to anterior end 15.1 mm. Ovoviviparous. Larvae freed through prolapse of uterus and body wall of anterior end of gravid individual.

First stage larva: Average length of 50 killed specimens 0.43 mm; of 15 living specimens 0.45 mm; cuticula striated; tail long and attenuated.

Second stage larva: Length of killed specimens 0.62–0.65 mm; cuticula smooth; tail short and notched with lobes laterally placed; several unicellular glands in head region and around rectum and in tail.

Definitive host: Thamnophis sirtalis Linnaeus.

Intermediate host: Cyclops viridis Jurine, 1820.

Experimental transfer host: Frog tadpoles.

Received for publication, February 14, 1938.

* A contribution from the University of Michigan Biological Station and the Department of Zoology of the University of Minnesota. The writer wishes to express his appreciation to Dr. W. A. Riley, Dr. L. J. Thomas, Dr. W. W. Cort, and the late Dr. F. N. Blanchard for help, criticisms, and suggestions.

Habitat: Adults in subcuticular connective tissue; developing specimens in serous membranes, connective tissue surrounding internal organs, and subcuticular tissue; larvae free in body cavity of intermediate host.

Type locality: Douglas Lake region, Cheboygan County, Michigan.

Cotypes: U. S. Nat. Mus. Helm. Coll. No. 9195.

The most reliable character for differentiation is the arrangement of the genital papillae of the male. This has been worked out for *D. medinensis, D. dahomensis* (Moorthy, 1937), and *D. ophidensis* and clearly separates these three species (figs. 1, 2 and 3). In addition, *D. ophidensis* differs from *D. dahomensis* in having the spicules proportionately longer. The spicules of *D. globocephalus* (Mackin, 1927) are distinctly unequal, thereby separating it from *D. ophidensis.* The spicules of *D. fuelleborni* (Travassos, 1934) are shorter than those of *D. ophidensis.* The exact status of *D. houdemeri* (Hsü, 1933) seems to be a matter of personal opinion. This species was based on the configuration of the anterior end of a single female specimen. Moorthy (1937) has not accepted this as sufficient evidence upon which to establish a species. As Hsü figures it, the cephalic aspect of *D. houdemeri* seems to be so strikingly different from that of all other forms that I am inclined to accept his species as valid pending further studies and evidence. *D. ophidensis* differs from *D. houdemeri* in the configuration of the cephalic aspect of the head of the female (figs. 5, 6, 7 and 8).

With Moorthy's work on *D. medinensis* and *D. dahomensis* and the present work on *D. ophidensis,* it becomes apparent that no adequate diagnosis of the genus exists, so the following revision is proposed.

Dracunculus Reichard, 1759, diagnosis emended

Dracunculidae. Anterior end with a circumoral elevation that is usually very prominent but variable in configuration. Mouth opening small. Inner circle of 4–6 papillae (interno-laterals always separate, interno-dorsals and/or interno-ventrals sometimes partially fused) and an external circle of 4 double papillae. Amphids posterior to lateral papillae. Deirids slightly posterior to nerve ring. Anterior muscular end of esophagus short. Glandular section long and divided into two parts, a short anterior section and a much longer posterior section separated by a constriction. Nerve ring encircling esophagus at this constriction. Intestine a straight tube. Fully matured female enormously longer than male.

Female: Vulva close to the middle of body, atrophied or closed in gravid individuals. Vagina runs forward for a short distance. Two uteri forming a continuous thin-walled tube, one anterior and one posterior to vulva, almost completely filling body of gravid specimens. Ovaries straight or the tips may be bent backward. Ovoviviparous.

Male: About 10–13 pairs of genital papillae, some preanal, others postanal, and variable in arrangement. Spicules subequal (distinctly unequal in one species), 0.38–0.73 mm, usually protruding about half their length. Gubernaculum present. Genital system a straight tube, testis and vas deferens merging imperceptibly into one another. Posterior end characteristically coiled into a tight spiral of about four loops.

Larva: Free swimming; 0.33–0.57 mm long; tail long and attenuated; develop to infective stage, which has a short, bilobed tail, in cyclops.

Adults: Parasitic in connective tissues of mammals and reptiles.

Type species: Dracunculus medinensis (Linnaeus, 1758).

LIFE CYCLE OF *Dracunculus ophidensis*

The adult worms in natural infections

Examinations of garter snakes (*Thamnophis sirtalis*) in the early part of July yielded gravid females of the parasite in which the embryos were completely developed and ready to emerge as the first stage larvae. It was not necessary to kill the snakes to determine the presence of an infection because gravid females produce visible swellings on the skin. Dissections showed these worms to be up to 25 cm in length with the posterior three-fourths coiled loosely on the surface of the back muscles and the anterior one-fourth imbedded in the subcutaneous connective tissue and partially disintegrated. While their linear distribution is not limited, they occur most often on the dorsal side of the host. It is not always true that the females migrate into the subcuticular tissue. An exception was noted on July 4, 1937, when a snake showing the dermal lesions was autopsied. Besides a specimen found in the usual location, four fully grown and gravid females were found within the body cavity coiled in the mesenteries. These specimens were entire, the anterior end not having disintegrated. No males were found in this case.

In not all of the infected snakes dissected were male specimens found, even though the females present were gravid. Exactly what becomes of the males is not known. They were found in several cases, but in all except one there were fewer males than females. Moorthy's experimental infections produced fewer males than females, especially if the infection extended into many months (Moorthy, 1937). When the males of *D. ophidensis* were found, they were in good condition, showing no signs of disintegration. They were coiled in the connective tissues usually between the body and the skin but one specimen was found in the pericardial membrane. Like the females, the males were not restricted to any particular region of the body. Many were close to the females. These observations relative to the males are significant, for certain earlier workers had either doubted the existence of males in this genus or argued that fertilization took place in the intestine of the host, the males never penetrating into the tissues with the females but passing out with the feces. With the discovery of the males of a number of species of *Dracunculus* (Neumann, 1895; Mackin, 1927; Moorthy and Sweet, 1936), these views are definitely disproven.

The snake host, as far as has been determined, is little injured by the presence of *D. ophidensis*. There is a certain amount of tissue response to the presence of the gravid females as has already been indicated. This response is in the form of a definite hypertrophy of the subcuticular connective tissue around the coiled anterior end of the worm causing the dermal elevations so characteristic of infection. The remains of a female

worm which has given off its larvae are in some way disposed of by the snake's tissues, for all traces of the infection disappear by fall or early winter. The dermal elevations with masses of disintegrating worms may remain for a time, but they disappear eventually leaving no external evidences.

The response of the gravid female of *D. ophidensis* to water is similar to that described for *D. medinensis.*

The first stage larva

Larvae from the gravid female freed into tap or lake water were very active. They moved constantly, bending their bodies back and forth in a stiff, wiry fashion. While the larvae are active, they remain in suspension in the water, not settling to the bottom. In this way, they differ from *Philometra* larvae, which adhere to the bottom or to some object with their tails and lash about with their free ends. They remain active for several days in lake water.

Morphologically, this stage is characterized by an exceedingly long and attenuated tail. Measurements of the living worms were made with difficulty because of their constant activity. The average length of fifteen larvae was 0.45 mm. This differed somewhat from the measurements of fifty killed larvae which averaged 0.43 mm long by 0.016 mm in diameter.

Infection of the copepod host

In the case of *D. ophidensis,* the cyclops stage of the life history proved to be an exceedingly easy one to demonstrate. Larvae were taken from a gravid female of an infected snake that had been in water for a short period, and when diluted a slight amount they were ready to be added to the cyclops cultures in the required quantity.

Cyclops were collected near the shore of Douglas Lake in front of the Biological Station. A tow net of relatively coarse mesh was used since it was desirable that smaller plankton escape. The plankton was distributed among a number of glass dishes so that there was at least one hundred cyclops in each dish. It was found to be unnecessary to isolate the cyclops from the rest of the plankton as the larvae were taken only by the cyclops. Several drops of the concentrated *Dracunculus* larvae were put into each dish and the plankton diluted with lake water a few hours later. In most of the cultures, two to five but sometimes as many as seven larvae were found in one cyclops and ordinarily not a single cyclops was found that did not contain at least one larva. Apparently, the cyclops were not inconvenienced by harboring the *Dracunculus* larvae, which maintained considerable activity within the body cavity of the cyclops for a few days, even making their way far out into the tail. When quiescent, the larvae were usually doubled once or twice on themselves.

In order to examine cyclops rapidly for the presence of larvae, they were drawn into a pipette and transferred to a glass slide in a drop of water. By carefully removing most of the water and leaving the cyclops stranded, they could be easily observed under low power of a compound microscope and the presence of larvae readily detected.

The second stage larva

It was found that the larvae molted between the 12th and 15th days after entering the cyclops. At this time the long attenuated tail was lost and the length was increased to 0.62 mm. The tail at this stage is characteristically notched or bilobed, the lobes being laterally arranged. The larvae became less active but were still seen to move about. However, they showed no indications of leaving the cyclops. When these larvae were mechanically freed from the cyclops, they moved rather sluggishly and tended to coil and uncoil rather than swim as did the first stage larvae.

There is some evidence to indicate that *D. ophidensis* is specific for its intermediate host. Larvae were found to develop readily in almost all *Cyclops viridis,* the form most frequently taken in plankton hauls. They were never found developing in other species, such as *C. serrulatus* Fischer, 1851, but only small numbers of *C. serrulatus* were examined.

Infection of the definitive host

Since no absolutely parasite free snakes were available, feeding experiments that completely prove the course of the life cycle of this parasite were impossible. However, the snakes were caught before the parasites were shedding larvae, so that the snakes probably had not contracted an infection that could be confused with the experimentally introduced worms.

The larvae were allowed to develop in the cyclops for at least 15 days before they were used in further experiments. One feeding consisting of about twenty infected cyclops was given to each of three snakes. Nematodes found in these snakes four months after this feeding have been definitely determined as *Dracunculus* identical with the adults from which the larvae were obtained. Both male and female specimens were found which were completely developed sexually but had not reached complete size development. The males were from 12–14 mm in length as compared with 16 mm, which is the length of males found with gravid females in June and July. The females were about 25 mm in length as compared with the 250 mm length of gravid individuals. One experimental garter snake harbored thirteen females and four males, the second had three females and four males, and the third had three females and one male. These worms were found in the loose connective tissues and serous membranes. About half of the specimens were localized around the heart and

along the tongue and esophagus, while the rest were distributed quite generally in the membranes lining the body cavity and in the subcutaneous tissues.

It is not known how many cyclops a garter snake may ingest, but some doubt is raised as to whether they take in enough to account for the high incidence of the infection that is seen in some localities. In examinations of a good many specimens, no naturally infected cyclops were found, so it seems that the incidence in this intermediate host is not great. The question then arises as to whether some other animal acts as an intermediate host to this form, or whether there exists some form which obtains the cyclops in greater numbers and is in turn eaten by snakes, thus acting as a transfer host. Tadpoles were thought to be the most likely form that could be involved, so an attempt was made to check this point.

Interpolation of the tadpole in the life cycle

In the summer of 1934 and again in 1936, it was observed that tadpoles would eat cyclops if they were put in the same dish. Further, if these cyclops contained the second stage larvae, the larvae with their characteristically notched tails were found free in the body cavity of the tadpoles at least two weeks after they had been ingested. Apparently, the larvae find a suitable environment in the tadpoles, but no indication of further growth or development was noted. In 1936, six tadpoles that had ingested infected cyclops were fed two each to two garter snakes and to one water snake (*Natrix sipidon*). Four months later these were examined. The first garter snake harbored thirty-five female and nine male specimens of *D. ophidensis,* the second was negative (it was in poor condition and had not eaten for a long time), and the water snake had only two females (see section below on host specificity).

This is the only evidence available to show the relationship of tadpoles to the life cycle. Tadpoles may serve as a transfer host but are not necessary and whether they are involved in nature cannot be concluded.

Tadpoles would ingest great numbers of the first stage larvae that could be found still active within the intestine on the following day. However, they had all disappeared within a week. It was concluded that the tadpole could not serve as an intermediate host for the development of the larvae to the infective stage.

Incidence, host specificity and distribution of the infection

D. ophidensis has been found in snakes from a number of localities in the Douglas Lake region, some of them quite widely separated. In 1934, thirty-nine garter snakes from the immediate vicinity of the biological station were examined, and seventeen, or 44%, were harboring this nematode. In 1935 and 1936, the worms seemed to occur about as frequently

as they did in 1934. In 1937, the occurrence of the parasite was determined by a superficial examination of living snakes for the gravid females under the skin. With such a procedure some cases may have been overlooked and some other snakes may have entered the tabulation twice if they were accidentally captured a second time. Of ninety-three garter snakes examined only five showed indications of the infection.

Three species of snakes in the Douglas Lake region (*Thamnophis sirtalis, T. sauritas,* and *Natrix sipidon*) are more or less similar in their habitats and habits. Because of this it is significant to compare the occurrence of the parasites in them. *Dracunculus* was found in one *N. sipidon* in 1934 and not seen again in this species until 1937 when one out of fifty-three was found to be infected. Fully as many ribbon snakes as garter snakes from the same areas have been examined but none has been found infected. Apparently the garter snake is the most suitable host for this parasite.

Of sixteen garter snakes and fourteen water snakes collected in late May, 1937, in the valley of the Minnesota River near Minneapolis, *Dracunculus ophidensis* was found in one of the garter snakes and three of the water snakes. This suggestion of widespread distribution is supported in the finding of these worms in a water snake from Ithaca, New York (Elmer Brown, personal communication). It is interesting to note that in this group of snakes from Minnesota the parasite was more prevalent in the water snakes which is contrary to what was found in the Douglas Lake area.

SUMMARY

A nematode parasite, *Dracunculus ophidensis* n. sp., of the garter snake is described and compared with the five other species of *Dracunculus* that are considered valid. The genus *Dracunculus* is redescribed.

Natural infections of *D. ophidensis* are described and its pathogenicity discussed.

Larvae were experimentally found to develop in *Cyclops viridis* and to reach the infective stage in 12–15 days. The first and second stage larvae are described.

Snakes became infected with *D. ophidensis* upon ingestion of these infected cyclops.

Tadpoles could be used as experimental transfer hosts and snakes became infected with *D. ophidensis* upon eating them. No development took place within the tadpole and it is not considered that they could serve as the sole intermediate host. It is not known whether tadpoles are involved in the life cycle in nature.

In the Douglas Lake region *Thamnophis sirtalis* seems to be the most suitable definitive host and *Cyclops viridis* the most suitable intermediate host.

The incidence of the infection in garter snakes was observed to reach 44% in one locality one season. The species has been recorded from Michigan and Minnesota.

Bibliography

Hsü, H. F. 1933 On *Dracunculus houdemeri* n. sp., *Dracunculus globocephalus,* and *Dracunculus medinensis.* Z. Parasitenk **6**: 101–118.

Mackin, J. G. 1927 *Dracunculus globocephalus* n. sp., from *Chelydra serpentina.* J. Parasitol. **14**: 91–94.

Moorthy, V. N. 1937 A description of *Dracunculus medinensis.* J. Parasitol. **23**: 220–224.

Moorthy, V. N. and Sweet, W. C. 1936 A note on the experimental infection of dogs with dracontiasis. Indian Med. Gaz. **71**: 437–451.

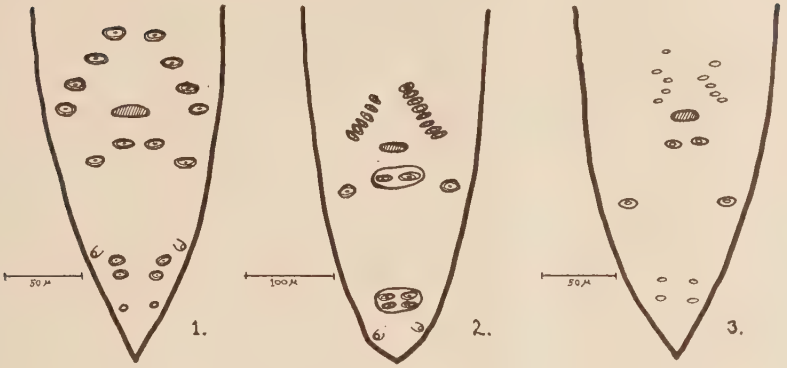
Neumann, G. 1895 Sur une filaire (*Filaria dahomensis,* n. sp.) du Python de Natal, voisine de la filaire de Medine. Bull. Soc. Zool. France **20**: 123–127.

Travassos, L. 1934 *Dracunculus fuelleborni* sp. n., in subcutaneous connective tissue of *Didelphus aurita* (Brazil). Mem. Inst. Oswaldo Cruz **28**: 235–238.

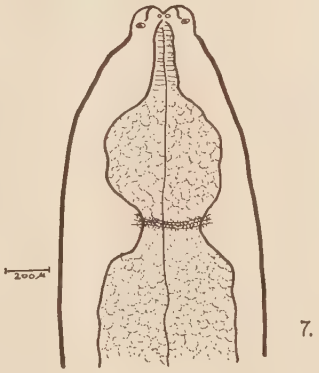
Explanation of Plate

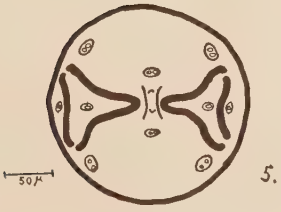
All Drawings Semi-Diagrammatic

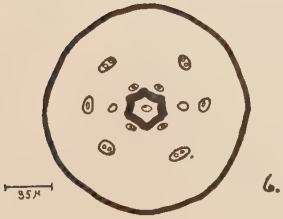
Fig. 1. Tail of *Dracunculus medinensis,* ventral aspect (from Moorthy, 1937).
Fig. 2. Tail of *Dracunculus dahomensis,* ventral aspect (from Moorthy, 1937).
Fig. 3. Tail of *Dracunculus ophidensis,* ventral aspect.
Fig. 4. Tail of *Dracunculus ophidensis,* lateral aspect.
Fig. 5. Head of female *Dracunculus houdemeri,* en face (from Hsü, 1933).
Fig. 6. Head of female *Dracunculus ophidensis,* en face.
Fig. 7. Head of female *Dracunculus houdemeri,* lateral aspect (from Hsü, 1933).
Fig. 8. Head of male *Dracunculus ophidensis,* lateral aspect.

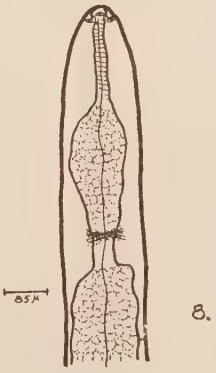

50 μ
1.
100 μ
2.
50 μ
3.


100 μ
4.


200 μ
7.


50 μ
5.


35 μ
6.


85 μ
8.

SEASONAL DISTRIBUTION OF TICK PARASITES

C. L. Larson
Bureau of Biological Survey, U. S. Dept. of Agriculture
AND
R. G. Green
Dept. of Bacteriology and Immunology, University of Minnesota

During the spring and summer of 1935 an opportunity to study the seasonal distribution of the tick parasite (*Ixodiphagus texanus*) was afforded by the discovery of this parasite in rabbit ticks (*Haemaphysalis leporis-palustris*) in Minnesota. Larson (1937) reported the recovery of tick parasites from nymphal ticks taken from two snowshoe hares and a cottontail rabbit in Morrison County, and from a ruffed grouse in Pine County, Minnesota. Previous to these isolations it had been believed that parasites occurred in an active state in nymphal ticks only. It was thought that after oviposition in a larval tick, the eggs of the parasite lay dormant until the tick developed into an engorged nymph, a process known as "latency." In our studies we observed for the first time active parasitism of larval rabbit ticks by *I. texanus*.

The actual observations carried out on well established colonies of tick parasites have been limited because the number of areas are few in which such colonies are known to exist. Philip (1931) reported a large colony of *Hunterellus hookeri* in Africa. He first noted an adult parasite on a dog in Lagos, Nigeria, West Africa, in February, 1929, and later found the insects in large numbers on dogs from late March until June, 1929. During the two weeks previous to April 12, 1929, he collected 323 nymphal ticks of *Rhipecephalus sanguineus* from two dogs. These ticks he reared and observed for evidence of parasitism. Parasites emerged from 231 nymphs and were present in, but failed to emerge from 60 others. More than 90 per cent of the total number of ticks collected were infected with parasites. Philip stressed the apparent difficulty of establishing colonies of these parasites for tick control in temperate climates. Da Costa Lima (1915) reported a colony of *H. hookeri* among *R. sanguineus* nymphs taken from dogs in Brazil.

Cooley and Kohls (1934) working experimentally with *I. caucurtei* in *Dermacentor andersoni* showed that this parasite tends to emerge in Montana during late August and early September. The opportunity of the parasite to establish itself in Montana is markedly reduced by the scarcity of its most favorable host, the nymphal tick, during the period of emergence. The only means by which it could survive and increase would be by remaining in a latent phase in overwintering flat nymphal

Received for publication, February 18, 1938.

ticks resulting from the seeds present during the time of parasitic activity. This method, however, is unsatisfactory from the standpoint of the rapid establishment of a colony of parasites of sufficient magnitude to be a factor in tick control, as the mortality of unfed nymphs throughout the winter is tremendous. It was also apparent that only one generation of parasites could be obtained in the course of a tick season.

Our studies were undertaken in order to determine the seasonal distribution of *I. texanus* in Morrison County, Minnesota, the duration of their occurrence, and the stages of tick parasitized. Collections of ticks on snowshoe hares were made from the time ticks appeared in the spring until they disappeared in late fall. Larval and nymphal ticks were taken in nearly equal numbers, placed in rearing tubes and sent to Minneapolis, where they were stored and observed. No observations were made on ticks reared under natural conditions.

A total of 7566 engorged, immature rabbit ticks was collected from 139 snowshoe hares shot on the Lake Alexander Area from April 2, 1935, to November 22, 1935. Of these, 3115 were engorged nymphs and 4451 were engorged larvae or seeds. During April only 26 nymphs and 4 seeds were collected. Three nymphs, one of which was parasitized, were discovered on a hare on April 23. On another hare, shot on April 25, there were 17 nymphs of which 2 were parasitized, and 4 seeds from 3 of which parasites later emerged. These were the first parasitized ticks isolated during the year. While it is true that the actual number of ticks examined was small, the date of appearance of affected ticks can be approximated from the collections made.

During May and June 189 nymphs and 45 seeds were collected; 30 of the nymphs and 5 of the seeds were parasitized. In July, of 290 nymphs and 378 seeds 1.7 per cent and 1.3 per cent, respectively, were parasitized. During the next 3 months, the number of ticks examined was increased as there were more available. In August, 801 nymphs and 1336 seeds were stored. The degree of parasitism noted was 4.1 per cent for nymphs and 9.9 per cent for seeds. During September there was a decrease of nymphal parasitism to 2.6 per cent of 1149 ticks; but the larval infection remained high, 10.9 per cent of 180 seeds. An abrupt decrease in parasitism was seen in October when only 0.1 per cent of 603 nymphs and 0.7 per cent of 858 seeds were affected. In November, no parasites were found in any of the 57 nymphs and 178 seeds collected. The date of the last recovery of an affected nymph was October 3, and the corresponding date for larvae was October 5.

There were 102 parasitized nymphs present among 3115 nymphs or an infection of 3.3 per cent. Among the 4451 larvae, 332 or 7.4 per cent were parasitized. For the entire group, including both larvae and nymphs, the degree of parasitism was 5.7 per cent. The figures are sum-

marized in Table 1. It should be pointed out that the numbers of nymphs and seeds examined, as shown in the table, are no indication of the stage of tick constituting the greater part of the population at any one time, since nymphs and seeds were collected in approximately equal numbers regardless of their relative abundance.

TABLE 1.—*Summary of parasites collected from engorged larvae and nymphs*

Month	Number of hares	Number of engorged nymphs	Number parasitized	Percentage parasitized	Number of engorged seeds	Number parasitized	Percentage parasitized
April	7	26	3	11.5	4	3	75
May	20	82	12	14.6	14	4	28.5
June	16	107	18	16.8	31	1	3.3
July	18	290	5	1.7	378	5	1.3
August	22	801	33	4.1	1336	133	9.9
September ...	24	1149	30	2.6	1652	180	10.9
October	21	603	1	0.1	858	6	0.7
November ...	11	57	0	0.0	178	0	0.0
Totals ...	139	3115	102	3.3	4451	332	7.4

Tick populations have been followed by counting the number of ticks taken from hares shot on the Lake Alexander Area. In 1935 ticks began to appear in small numbers during the first two weeks in April and steadily increased in number until the first two weeks in June when an average population of 1700 ticks per hare was reached. A decline to approximately 1200 ticks per hare followed and persisted until the latter half of July when a marked increase in population again began. A peak population was maintained throughout August and September when the average infestation was 4200 ticks per hare. By the middle of October the number of ticks had decreased to 1400 and in early November they practically disappeared. The rise in tick population which occurred during April and early May was due primarily to the appearance of adults, but the further rise as the year advanced was attributable to increased numbers of emerging immature forms. On the Lake Alexander Area it would appear that both larval and nymphal ticks are present throughout the greater part of the tick season. During the earlier months nymphs predominate and during the later months larvae are much more numerous. When at a maximum the population is made up predominantly of larval ticks.

The occurrence of *I. texanus,* actively parasitizing both nymphs and seeds of the rabbit tick throughout the greater part of the tick season in Minnesota has been demonstrated. The fact that parasites were isolated from both seeds and nymphs taken from a snowshoe hare on April 24, 1935, soon after immature ticks made their appearance, indicates that these insects have the ability to overwinter in either engorged or unengorged immature ticks.

As the number of immature ticks increased, the cases of parasitism

likewise increased. In August and September when there were approximately 4,000 ticks per snowshoe hare, the incidence of affected ticks was greatest. In October the average infestation of ticks per hare decreased to 1,400, and the number of parasitized larvae and nymphs found among them decreased almost to the vanishing point. In spite of the fact that ticks persisted throughout November, no parasites were recovered during that month.

The degree of parasitism noted among nymphs and larvae of *H. leporispalustris* during any period of the tick season is conditioned by many interdependent factors. The factors most significant are: the ability of the parasites to overwinter in sufficient numbers to maintain themselves; the emergence and engorging of infected ticks at such a time that the resulting parasites emerge when an abundance of suitable hosts is available; and the stages and numbers of ticks prevalent when adult parasites are active. The insects seem to be able to overwinter successfully in both nymphs and seeds, as is shown by their early recovery from these stages. Inasmuch as overwintering adult ticks fail to emerge early enough to produce larvae in April, it is assumed that the seeds collected at that time overwintered as such. As is evident from the data we have compiled, infected immature ticks are active throughout the early part of the season, so that the immediate development of parasites is possible. The parasitized ticks recovered from April until the latter part of July are those in which oviposition had occurred the previous year. During these months in 1935, 7.5 per cent of nymphs and 3.0 per cent of larvae observed were parasitized. The comparatively greater number of parasitized nymphs found in the spring may hardly be accounted for merely on a basis of the relative death rates for overwintering nymphs and larvae.

It may be that the phenomenon of latency plays some rôle in the increase in nymphal parasitism. The parasitism of larger percentages of seeds became apparent in August and September. The figures of larval infection rose to 9.9 per cent in August and increased further in September to 10.9 per cent. Among the nymphs we found a 4.1 per cent infection in August and a decrease to 2.6 per cent in September. August and September are the months in which adult parasites emerge. As the number of nymphs at this phase of the tick season has decreased to such an extent that they constitute only a small part of the tick population of the area it is not surprising to note the relatively low percentage of parasitism of nymphs in comparison with the percentage of larval infection. As parasites oviposit at random, the stages of tick making up the greater part of the population will most often be selected. Even though the tick population was still large in October and November, instances of infection were few, as adult parasites were no longer present.

Cooley (1928) has shown that *I. caucurtei* emerges in from 40 to

51 days at a constant temperature of 22° C. The emergence of these insects is delayed until late summer when they are placed under natural conditions such as prevail near Hamilton, Montana. The dates of emergence were closely grouped in all samples of parasitized ticks released, no matter how early or how late they were placed in the natural environs. If, as our collections show, *I. texanus* emerges in greatest number in August and September, the possibility of the production of more than one generation a year is remote. A few parasites emerging early might produce another generation the same season, but the number of cases in which this could occur would be small. Failure to complete more than one generation a year materially decreases the efficiency of the parasite as a factor in the biological control of ticks. Since the parasites emerge early enough in the season to account for a 4.1 per cent incidence of nymphal parasitism in August, it is possible that their numbers may be increased over a period of years.

In some instances the number of parasites emerging from affected ticks was determined. The number found in larvae varied from 1 to 3 and averaged 2.1; the number in nymphs varied from 3 to 10 with an average of 5.4. The limiting factors are the size of the host, and the ratio of female parasites engaged in the act of oviposition to the hosts available for this purpose. Cooley and Kohls (*loc. cit.*) have shown that under experimental conditions 2 to 73 parasites (*I. caucurtei*), or an average of 21.2, emerged from nymphs of *Dermacentor andersoni*. The larger number in their series is accounted for by prevailing experimental conditions in which numerous female parasites were allowed to oviposit under ideal circumstances, and by the larger size of nymphs of *D. andersoni* as compared with nymphs of *H. leporis-palustris*.

The ratio of male to female parasites emerging from ticks was observed in a few cases. Among parasites emerging from seeds, the proportion was practically equal, while among those from nymphs we found a ratio of about five females to each male.

The incidence of parasitism of rabbit ticks by *I. texanus* is only 5.7 per cent for both nymphs and larvae. This low incidence indicates that at present the parasites are not active as a factor in the control of rabbit ticks on the area studied. Whether or not they are increasing and will later serve as a control factor is not yet apparent. It seems, however, that they are sufficiently well established to maintain the colony and to increase to such an extent that they may be of benefit. The parasite is widely distributed, as we have shown its presence in Lake of the Woods and Pine Counties as well as in Morrison County, Minnesota.

The possibility of introducing *I. texanus* into areas where it may be used as a control factor presents itself. Attempts to introduce other species of tick parasite have not been successful. As Philip pointed out,

H. hookeri, which is found in tropical climates, would be at a distinct disadvantage if transferred to temperate climates. *I. texanus,* however, has shown the ability to maintain itself in Minnesota, and should afford a more rugged strain which could be adapted for introduction into other temperate regions where biological control of ticks is desired.

SUMMARY

Between April 2 and November 22, 1935, 3115 nymphs and 4451 larvae were collected in Morrison County, Minnesota, reared and observed for signs of parasitism. Three and three-tenths per cent of the nymphs and 7.4 per cent of the larvae were infected. The parasitized immature rabbit ticks were found from April 23 to October 5, 1935. The incidence of parasitism was greatest during August and September.

Factors important in the parasitic involvement of immature ticks are described. This study indicates that the parasite is capable of producing but one generation a year.

In a group of infected ticks in which the parasites were counted, an average of 5.4 emerge from the nymphs and 2.1 from the larvae. The sex ratio of parasites emerging from seeds is approximately one to one, whereas the ratio of females to males among parasites emerging from nymphs is five to one.

The effect of *I. texanus* as a biological factor among the ticks on the area studied does not appear to be of great significance.

BIBLIOGRAPHY

COOLEY, R. A. 1928 Preliminary report on the tick parasite, *Ixodiphagus caucurtei* du Buysson. 7th Biennial Rep., Montana State Bd. Entom., pp. 17–31.

COOLEY, R. A., AND KOHLS, G. M. 1934 A summary of tick parasites. Proc. 5th Pacific Sc. Cong. **5**: 3375–3387.

DA COSTA LIMA, A. 1915 O. chalcideo *Hunterellus hookeri* Howard, parasite da carra pato, *Rhipicephalus sanguineus* Latrielle, observado no Rio de Janeiro. Rev. Vet. Zootechnia **5**: 201–203.

LARSON, C. L. 1937 The tick parasite, *Ixodiphagus texanus,* in nymphs and larvae of *Haemaphysalis leporis-palustris* in Minnesota. J. Parasitol. **23**: 496–498.

PHILIP, C. B. 1931 Occurrence of a colony of the tick parasite, *Hunterellus hookeri* Howard, in West Africa. Pub. Health Rep., U. S. Pub. Health Service **46**: 2168–2172.

MESOCERCARIA INTERMEDIA N. SP. (TREMATODA: STRIGEATA) WITH A NOTE ON ITS FURTHER DEVELOPMENT*

Louis Olivier and Theron O. Odlaug

During the summer of 1937 one of us (L. O.) collected hitherto undescribed mesocercariae from frogs and snakes in the Douglas Lake region in Michigan. These larvae were tentatively identified as mesocercariae of *Alaria mustelae* Bosma, 1931, but detailed study of the general morphology and of the excretory system disclosed significant differences between them and the description given by Bosma (1934).

During the same period, the second author had been conducting successful life history studies on mesocercariae from frogs collected in Vermont. Subsequent comparison of these larvae with those from Douglas Lake showed them to be specifically identical. Since they cannot be referred to any previously described species, the name *Mesocercaria intermedia* is proposed for them. In addition to the description of the mesocercaria, it is possible to add a preliminary note on the life history of the species. The writers are indebted to Dr. H. W. Stunkard for helpful suggestions during the preparation of the manuscript and to Dr. W. W. Cort under whose direction the studies at Douglas Lake were made.

Bosma (1934) pointed out that "The term agamodistomum simply means larval distome and is applicable to any immature distome without implication as to its possible systematic position" and she proposed the term mesocercaria to designate the "definite prolonged intermediate stage which occurs in the secondary host between the cercarial and the definitive metacercarial phases in the life cycle of those trematodes having four hosts." The term mesocercaria, proposed by Bosma for a developmental stage, may appropriately be used as the name for a larval group. Accordingly, we have employed *Mesocercaria* to supplant *Agamodistomum* as the name for the mesocercariae of trematodes with four-host life cycles.

Hughes (1928) described *Mesocercaria* (= *Agamodistomum*) *la-ruei* and listed all the species of mesocercariae known at that time. Lutz (1933a) discussed the known mesocercariae at some length and (1933b) figured a mesocercaria which he obtained by infecting tadpoles of toads and tree frogs with *Dicranocercaria gyrinipeta*. The first description of the excretory system of a mesocercaria was that of Cort (1918) for *Mesocercaria* (= *Agamodistomum*) *marcianae*. Cort and Brooks (1928)

Received for publication, February 18, 1938.

* Contribution from the Department of Biology, New York University, University Heights and the University of Michigan Biological Station.

described the cercaria that develops into *M. marcianae* in tadpoles. Bosma (1934) pointed out that the excretory system of the mesocercaria of *Alaria mustelae* is much simpler than that of *M. marcianae*.

The excretory system and most of the other morphological details of *M. intermedia* were worked out from living larvae flattened under a cover slip. Specimens of *M. intermedia* used for making measurements were fixed in hot corrosive acetic without pressure.

Mesocercaria intermedia n. sp.

(Fig. 1)

Specific description: Larvae either free or encysted in the muscles and pericardial region of tadpoles and adults of *Rana pipiens* Shr. and in fatty tissue of *Thamnophis sirtalis* L. In the snake the larvae accumulate in the tail. Anterior part of the worm covered with minute spines back to level of pharynx on elongated specimens. Spines close together around oral opening, more scattered near their posterior limits. Large, almost spherical acetabulum near middle of body, presenting, when expanded, a single row of minute spines. Average diameter of acetabulum of fifty mounted specimens is 0.059 ± .0004 mm. Oral sucker resembles that of typical strigeid carcariae, in fifty mounted specimens averaging 0.076 ± .0004 mm long by 0.052 ± .0004 mm wide. Short prepharynx, broad esophagus leading to large, thin-walled intestinal ceca. In flattened specimens, fig. 1, saccate ends of ceca lie median to and somewhat behind posterior pair of penetration glands. Two pairs of penetration glands, one pair in front of and the other pair lateral to the acetabulum, ventral to level of ceca. Ducts from the two glands on each side lie together and traverse the oral sucker where they are usually dilated. Gross structures absent from posterior third of worm. Arrangement of collecting tubules, capillaries, and flame cells in this region readily determined. Genital primordium, a small irregular mass of cells, in this area.

Excretory system: The excretory system of *Mesocercaria intermedia* was completely worked out by one of us (L. O.) and later confirmed by the other. The excretory pore is sub-terminal and the bladder is V-shaped. Each arm of the bladder narrows gradually and is continued anteriorly as a large common collecting duct which extends about two thirds of the distance to the acetabulum where it receives an anterior and a posterior main collecting tube. Each anterior main collecting tube receives separately, and at different levels, three branches, each of which in turn receives two accessory collecting tubules. The capillaries from four flame cells empty into each accessory collecting tubule at its distal end. Each posterior main collecting tube coils several times and then continues posteriorly to receive separately two branches, each of which receives two accessory collecting tubules with their respective complements of four capillaries and flame cells. Thus, there is a total of ten accessory collecting tubules and forty flame cells on either side of the body (fig. 1). The accessory collecting tubules are arranged in pairs, one of each pair is dorsal and the other ventral, thus, half the flame cells are dorsal and half ventral. Each posterior main collecting tube, after receiving the branch from the last group of

TABLE I

	Host I *R. pipiens* (adult) 50 larvae from cysts	Host II *R. pipiens* (adult) 25 larvae free in tissues	Host III *Thamnophis sirtalis* 50 larvae free in tissues	Host IV *Thamnophis sirtalis* 33 larvae free in tissues
Length Range ...	0.30 −.44 mm	0.33 −.51 mm	0.23 −.41 mm	0.27 −.43 mm
Mean ± P.E.	0.382 ± .003 mm	0.410 ± .007 mm	0.311 ± .004 mm	0.340 ± .004 mm
Width Range	0.12 −.19 mm	0.13 −.18 mm	0.11 −.16 mm	0.12 −.21 mm
Mean ± P.E.	0.158 ± .001 mm	0.156 ± .002 mm	0.140 ± .001 mm	0.150 ± .002 mm

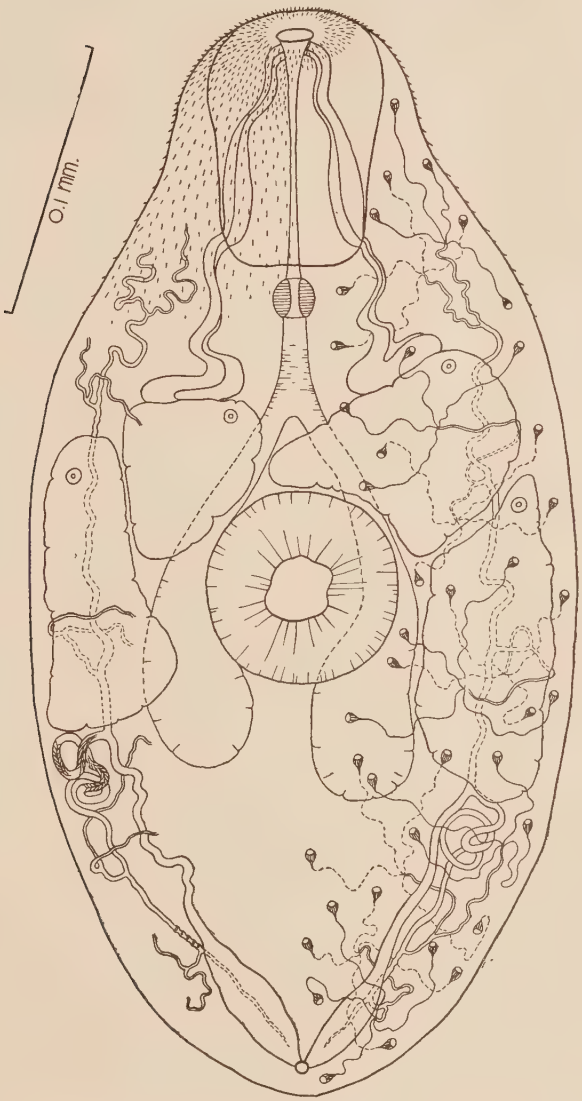


FIGURE 1. Ventral view of *Mesocercaria intermedia* drawn from living specimens. The complete flame cell pattern is shown on one side, capillaries and flame cells are omitted on the other side. Capillaries of the dorsal flame cells are shown with broken lines. Note cilia in the proximal portion of the posterior main collecting tube on the left. Complete spination is shown only on one side.

eight flame cells, runs back and terminates blindly dorsal to the bladder near the excretory pore. It is suggested that this structure may be the remnant of the tubule which in the cercarial stage led to the flame cell or flame cells of the tail. The proximal coiled portion of the posterior main collecting tube is ciliated in three places as shown in the figure. These ciliated patches are clearly separated from one another and the long cilia beat the fluid in the tube toward the bladder. The distribution of the branches on the main collecting tubes and the details of the distribution of the capillaries and flame cells are shown in the figure.

DISCUSSION

A large number of specimens of *M. intermedia* from four different individuals representing the two host genera were measured. The accompanying table presents the ranges and averages for the lengths and widths of the worms from each. When these data are treated statistically, it appears that the mean length of the specimens from each host differs significantly from that of the specimens from the other three. In addition, the mean width of the worms from host III appears to be significantly different from that of the worms from the other three hosts. While these statistical differences in size may indicate specific differences in the larvae from the different hosts, nevertheless the larvae are morphologically identical in all respects save size. Consequently, the data are interpreted to mean that the larvae represent but one species which varies in size depending on environmental and developmental factors. Perhaps the chief reason for the observed variations is that the several hosts had infections of worms which entered the tissues at different times. Thus, one may have had many larvae in early stages of growth whereas another had many fully grown worms. If this is true, the sizes of the worms in the different hosts are not comparable. It is concluded that size measurements are of little or no value for distinguishing species of mesocercariae collected from naturally infected hosts. Cort and Brackett (1938) showed that *Diplostomulum ranae* may vary considerably in size depending on the length of time the larvae have been in the host, and they suggested that "too much weight cannot be given to measurements of diplostomula as specific characters."

The excretory systems of *M. marcianae, M. intermedia,* and the mesocercaria of *Alaria mustelae,* though different in detail, are strikingly alike in their fundamental structure. In all three the excretory bladder is V-shaped and each arm of the bladder receives an anterior and posterior main collecting tube at a level posterior to the level of the acetabulum. In each case the flame cells are arranged in five equal groups on each side, three on the anterior main collecting tube and two on the posterior main collecting tube. The excretory systems of the species differ principally in the number of flame cells in each of the five groups. Because of this difference it is possible to distinguish the three species on the basis of their excretory patterns alone. In the mesocercaria of *Alaria mustelae*

there are three flame cells in each of the five groups and consequently fifteen flame cells on each side. In *M. intermedia* there are two sets of four flame cells in each group and forty flame cells on each side. In *M. marcianae* there are two sets of six flame cells in each group and sixty flame cells on each side. Thus, in the three species there is a progressive increase in the number of flame cells in the primary groups, with *M. intermedia* as an intermediate stage between the other two. Presumably the flame cell systems of all three species were derived from the same basic pattern (see Cort and Brooks, 1928). The excretory system of *M. intermedia* differs from the others in two additional details of structure. In this species the proximal portion of the posterior main collecting tube is ciliated and distally it terminates blindly dorsal to the bladder. This blind tube persists in old and encysted specimens. Such a tube was not described for either the mesocercaria of *Alaria mustelae* or for *M. marcianae* and Olivier was unable to find it in specimens of *M. marcianae* examined during the summer of 1937.

It is also possible to distinguish the three mesocercariae on the basis of other structural features. Each has four penetration glands (Lutz has reported a species of mesocercaria with eight) but in *M. marcianae* they are located more anteriad than in the other two species. In addition, *M. marcianae* differs from the others in that the entire ventral surface bears scattered spines. The extent of spination, the position and number of the penetration glands, and the structure of the excretory system are the best criteria for distinguishing species of mesocercariae.

The three mesocercariae which have been discussed are so similar in their fundamental structure that they must be closely related. As a consequence of their close morphological agreement, it might be expected that both *M. marcianae* and *M. intermedia* develop into adults in the genus *Alaria* and have four-host life cycles. Actually, one of us (T. O.) has been able, by feeding *M. intermedia* to rats, to obtain metacercariae which become adults in the intestine of cats. The adults belong to the genus *Alaria*. (The life history of this form will be described in detail elsewhere by T. O. Odlaug.) The further development of *M. marcianae* has not been reported.

No attempt is made at this time to compare *M. intermedia* with other previously described mesocercariae because their descriptions are too incomplete.

SUMMARY

Mesocercaria intermedia is described as a new species from frogs and snakes. The excretory system is described in detail and compared with that of other mesocercariae. The life history of *M. intermedia* is similar to that of *Alaria mustelae,* and a preliminary account of it is given.

Bibliography

Bosma, N. J. 1934 The life history of the trematode *Alaria mustelae* Bosma, 1931. Tr. Am. Micr. Soc. **53**: 116–153.

Cort, W. W. 1918 The excretory system of *Agamodistomum marcianae* (La Rue), the agamodistomum stage of a fork-tailed cercaria. J. Parasitol. **4**: 130–134.

Cort, W. W. and S. Brackett. 1938 A new strigeid cercaria which produces a bloat disease of tadpoles. J. Parasitol. **24**: 263–271.

Cort, W. W. and S. T. Brooks. 1928 Studies on the holostome cercariae from Douglas Lake, Michigan. Tr. Am. Micr. Soc. **47**: 179–221.

Hughes, R. C. 1928 Studies on the trematode family Strigeidae (Holostomidae) XII. *Agamodistomum la-ruei* sp. nov. Parasitology **20**: 413–420.

Lutz, A. 1933a Consideracoes sobre o *Distomum tetracystis* Gastaldi e formas semelhantes, erroneamente chamadas agamodistomes. Mem. Inst. Oswaldo Cruz **27**: 33–60.

————1933b Notas sobre Dicranocercarias brazilieras. Mem. Inst. Oswaldo Cruz **27**: 349–402.

RESEARCH NOTES

CAPILLARIA INFECTION IN FISH

While examining the intestinal contents of fishes used in the biological control of dracontiasis, it was observed that some of them were naturally infected with an early-stage capillarid larva. A brief account of the larva is given in this paper.

Of the several species of copepodocidal fish examined from different freshwater ponds in the Chitaldrug District, Mysore State, India, only *Barbus puckelli* (62 %), *Barbus ticto* (22 %), and *Lepidocephalichthys thermalis* (15 %) have been found naturally infected with the *Capillaria* larva, which was usually found imbedded in the mucous coat or encysted in the peritoneal layer of the midgut.

The description and measurements given are of the larva preserved, stained, and mounted by the author's method (Moorthy, 1937, J. Parasitol., 23: 100–102).

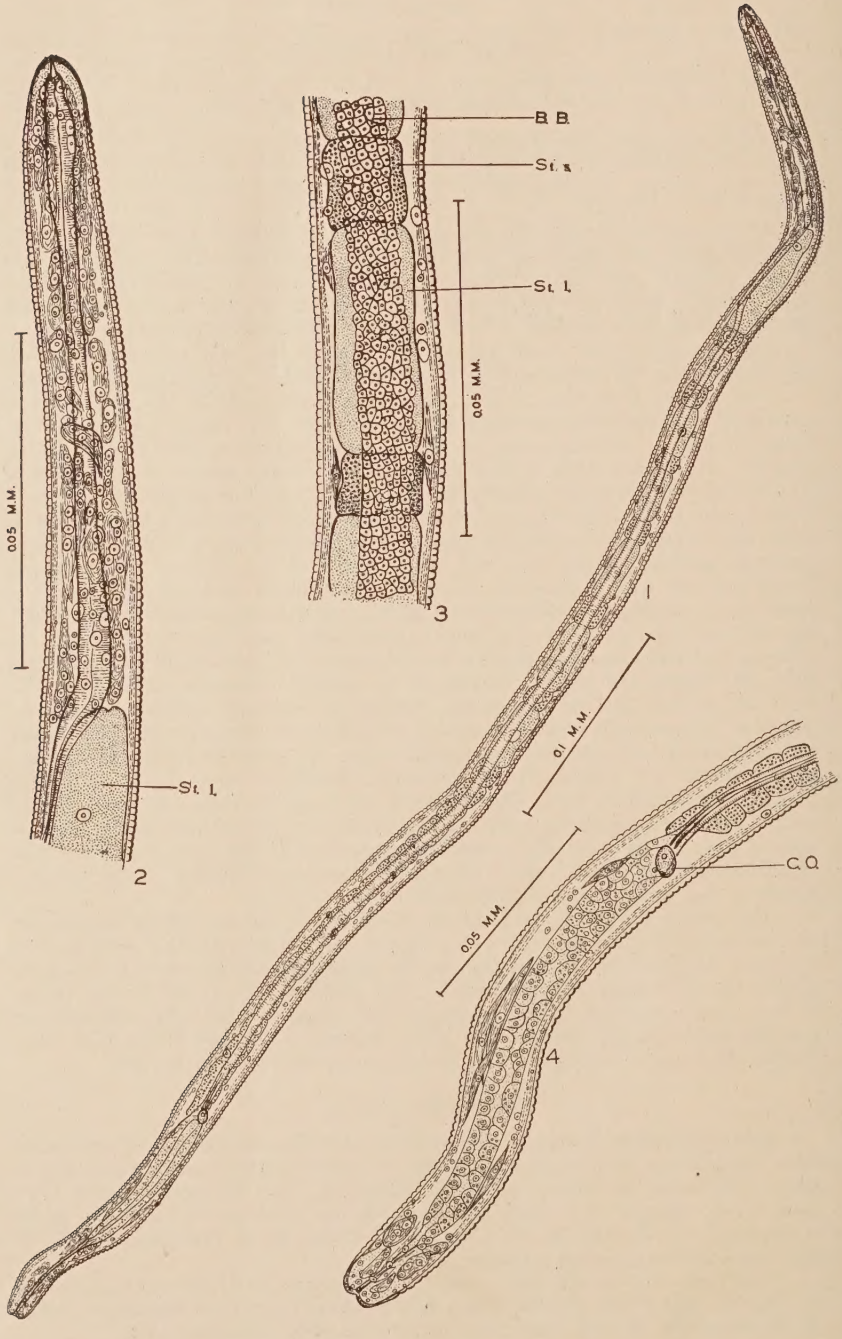
Description: Body, 0.60 to 0.76 mm long by 0.020 to 0.024 mm wide; narrow anteriorly and widening gradually posteriorly (fig. 1). Spear present; imperfectly chitinized, its length equal to about one-third body width in spear region (fig. 2). Nerve ring 0.052 to 0.070 mm from anterior extremity. Esophagus 0.50 to 0.62 mm long, occupying nearly six-sevenths of anterior portion of body and lying to one side of stichosome for a short distance anteriorly and then superficial to it (fig. 1). Esophageal lumen very narrow and indistinct anteriorly, widening gradually posteriorly and very distinct at its junction with intestine. Prominent cell with a conspicuous nucleus, probably a coelomocyte, present at junction of esophagus with intestine (fig. 4). Stichosome consisting of a single row of cells 0.46 to 0.55 mm in length, commencing about 0.05 to 0.10 mm from anterior end and extending slightly beyond junction of esophagus with intestine; first cell longest and slightly bilobed at its anterior extremity (figs. 1 and 2). Cells in anterior half of stichosome, varying in size and nature of contained granules; shorter cells containing coarse granules regularly alternate with longer cells containing comparatively fine granules (fig. 3); posteriorly the cells are uniformly small and contain coarse granules. Bacillary band lateral, extending along entire length of larva (fig. 3), its width equal to about one-third that of body. Intestine very short, being only about one-seventh of body length; it consists of large numbers of cells containing transparent globules and granules. Rectum 0.034 to 0.050 mm in length, thin-walled, narrow and tubular. Anus terminal. Posterior end rounded and slightly bilobed.

The size of the larva, the presence of single row of cells in the stichosome, the anterior portion of the body being considerably longer than the posterior portion, and the imperfect chitinization of the spear suggest that the larva is probably in the third stage of development. The presence of only third stage larvae, in some cases encysted in the intestinal wall, suggests that copepodocidal fish possibly act as second intermediate hosts; perhaps a copepod is the first intermediate host and a predacious fish, bird, or turtle is the definitive host.

Acknowledgment

Acknowledgment is made to the International Health Division of the Rockefeller Foundation for having granted the fellowship which made this work possible. The writer is indebted to Dr. B. Schwartz, Chief of the Zoological Division, U. S. Bureau of Animal Industry, for the privileges of working in his laboratory; and to Drs. W. W. Cort, E. E. Wehr, B. G. Chitwood, and G. F. Otto for valuable help and guidance.

This paper is a contribution from the Department of Helminthology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.—
V. N. Moorthy, *Mysore State Department of Health, Bangalore, India.*


B. B.
St. 2
St. 1.
0.05 M.M.
3
0.05 M.M.
St. 1.
2
1
0.1 M.M.
0.05 M.M.
C. Q.
4

Capillaria larva

FIG. 1. Median view.
FIG. 2. Anterior portion of the esophageal region.
FIG. 3. Lateral view; shows the bacillary band, the short and long stichosome cells.
FIG. 4. Intestinal region, lateral view.

Abbreviations: St.l. = Long cell of stichosome
St.s. = Small cell of stichosome
C.O. = Coelomocyte
B.B. = Bacillary band

A MICROSPORIDIAN PARASITIC IN *RETICULITERMES FLAVIPES*

In two workers of a colony of *Reticulitermes flavipes* collected for classwork in protozoology in May, 1937, from the vicinity of Urbana, Ill., a few spores of a microsporidian were observed. Repeated examination of the termites obtained from the same locality has failed to add any new material. In view of the fact that a single species of microsporidia, *Duboscqia legeri* Pérez (1908) parasitic in the body cavity of *Termes lucifugus*, is known up to the present time and further in view of the rare occurrence of the present microsporidian in this locality, I am writing this note with the hope that additional information may be obtained elsewhere.

In the fresh smears of the two host intestines, the spores were noticed in what appeared to be an adipose tissue situated close to the mid-intestine. As the other portions of the host bodies were discarded before being examined, the exact seat of infection is not known. The spores (Fig. 1) were scattered within the host cells without order, which suggests that the organism is probably a

FIG. 1. Three fresh spores of the microsporidian. ×2250.

species of *Nosema*. The spores are ovoidal in form and circular in cross-section. They are refractile and at one extremity a clear ovoid vacuole is regularly present, which is common among microsporidian spores. The comparatively large sporoplasm extends partially into this vacuole. Application of mechanical pressure brings about extrusion of the polar filament which measures about 50 μ in length. In its structure, the spore resembles closely that of *Nosema aedis* Kudo or *Plistophora macrospora* Léger et Hesse. The fresh spores viewed in physiological solution measure 6.2–6.7 μ long by 4.2–4.7 μ broad. The oval spores of *Duboscqia legeri* measured, according to Pérez, 5 μ long by 2 μ wide. Comparison of the spores of various species of microsporidia in my collection and of published descriptions of other species, with the present form leads me to consider the microsporidian under notice as new to science.—R. R. KUDO, *Department of Zoology, The University of Illinois, Urbana, Ill.*

METACERCARIAE OF A SPECIES OF *BRACHYLAEMUS*, PROBABLY *B. VIRGINIANUS*, FROM *AGRIOLIMAX AGRESTIS*

Various terrestrial STYLOMMATOPHORA serve as intermediate hosts for trematodes. Dolfuss (1935, Ann. Parasitol. **13**: 176–190) has given a very complete list of the reported cases.

In the family LIMACIDAE, larval stages of *Brachylaemus* have been found in

Lehmania marginata (O. F. M.), *Malacolimax tenellus* (Nilsson) and *Agriolimax agrestis* (Linn.). Infections in *A. agrestis* have been reported from Europe by Dujardin (1845, Hist. Nouv. Helm. ou Vers Intes. Nouv. suit à Buffon. Paris. **16**: 654), Baer (1928, Rev. Suisse Zool. **35**: 27–41), Henkel (1931, Z. Parasitenk. **3**: 664–712), and Joyeux, Baer, and Timon-David (1932, Compt. Rend. Soc. Biol. **109**: 464–466). In many cases it is impossible to know what species was involved.

In America, Sinitsin (1931, Z. Parasitenk. **3**: 786–835) described various larval stages of *B. virginianus* (*Harmostomum migrans*) from *Polygyra thyroides*. Krull (1935, Tr. Am. Micr. Soc. **54**: 118–134) showed by carefully planned experiments that *B. virginianus* will develop through all of its larval stages in *Polygyra thyroides*. Krull (1936, Proc. Helm. Soc. Wash. **3**: 56–58) added *Helix pomatia, Deroceras laeve,* and *Pseudosuccinea columella* as second intermediate hosts of *B. virginianus* and stated that species of *Helisoma* and *Succinea* may serve in that capacity.

Apparently larval stages of *Brachylaemus* have not been reported from *Agriolimax agrestis* in America. This paper is concerned with such a report. In the course of examining 200 of these snails, one was found to be infected with three metacercariae. The average dimensions of specimens fixed in Beauchamp's aceto-formol-alcohol (not under pressure) are: length 0.98 mm, width 0.40 mm, diameter of oral sucker 0.17 mm, acetabulum 0.14 mm, pharynx 0.09 mm. Other morphological characters are essentially like those given for the metacercariae of *B. virginianus* by Krull (1935).

Since there were no sporocysts or cercariae present in this snail, it is obvious that the metacercariae were acquired as a result of contact with slime or a snail bearing cercariae. It is not known whether the infection originated from the same, or a different species of mollusk.

The only *Brachylaemus* recorded from this vicinity (Charlottesville, Virginia) is *B. virginianus* from *Didelphis virginiana. B. peromysci,* a new species from *Peromyscus leucopus leucopus,* is reported by the author (1938, J. Parasitol. **24**: 245–248), but in this species the acetabulum is larger than the oral sucker. The reverse is true in the metacercariae found in *Agriolimax.* It seems highly probable, therefore, that *A. agrestis* may serve as a second intermediate host of *B. virginianus.*—Bruce D. Reynolds, *Miller School of Biology, University of Virginia.*

A NEW HOST AND LOCALITY RECORD FOR *SARCOCYSTIS RILEYI* (STILES, 1893)

Reports of *Sarcocystis* from ducks are extremely rare in the literature. C. V. Riley's (1869) report is probably the first authentic one, but in the intervening 69 years only half a dozen reports of this organism from wild ducks have been made. Previous reports from North America have recorded the following hosts: mallard (*Anas platyrhynchos*), shoveller (*Spatula clypeata*), and domestic duck. Infections have been previously recorded from Missouri, Minnesota, and the eastern seaboard, but since ducks are highly migratory, locality probably means very little.

During the past two years I have examined seven ducks heavily infected with *Sarcocystis rileyi.* These ducks had been shot by hunters, and were all apparently normal in flight and external appearance. In no case did the hunter report noticing anything unusual about the duck until preparing it for eating. So far as had been learned no hunter has bagged more than one infected duck, and each specimen has come from a different locality in the state.

An examination of these ducks revealed a rather heavy infection of *Sarcocystis.* In two cases the infection was so extensive as to extend to the head muscles. In general the cysts were concentrated in the superficial muscles of the breast, but cysts were found all through the skeletal muscles. In six of the seven cases examined the host was positively identified as *Anas rubripes,* the black duck, thus constituting a new host record. However, it is highly probable that this organism exhibits little or no host specificity.—Carl Gower, *Game Division, Michigan Department of Conservation.*